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Jervis's Insects as Natural Enemies: Practical Perspectives

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Formerly Edited by Mark A. Jervis

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Cover Illustration: Robber fly (Diptera: Asilidae) feeding on a phorid (Diptera: Phoridae). Photographer: Bernard D Roitberg, Professor Emeritus, Behavioral Ecology Research Group and Centre For Pest Management Department of Biosciences Simon Fraser University Burnaby BC Canada V5A 1S6 roitberg@sfu.ca

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To the memory of Mark Jervis and to all other natural enemy biologists, past, present and future

Mark A. Jervis during the 2006 Behavioural Ecology of Insect Parasitoids (BEPAR) meeting in Antibes, France (Photograph by George E. Heimpel).

Preface to the Third Edition

This book is not entirely as it should be. In a more ideal world Mark A. Jervis would have been an active contributor to this edition of his book, having co-edited the first edition (Jervis and Kidd, 1996) and sole-edited the second edition (Jervis, 2005). Mark died unexpectedly in March 2014. In his honour and memory, we edited a special issue of the journal *Entomologia Experimentalis et Applicata* (Hardy and Wajnberg, 2016a). That endeavour led to the idea that it might be due time for another edition of Mark's respected and valuable instructional volume on insects as natural enemies. We duly proceeded, with the full consent of Mark's family, and here it is.

This book is not, primarily, another memorial to Mark. The special issue serves that purpose more directly and our personal recollections of our association with Mark can be found within it (Hardy and Wajnberg, 2016b). What this book is, we hope, is a vibrant, continuation of Mark's vision for the type of resource that is needed by researchers studying insects as natural enemies, both in terms of fundamental research and of agro-ecological applications, as outlined in the prefaces to the previous editions. In the preface to the first edition it was mentioned that '*Statistical aspects of sampling and experimental design are hardly gone into*': we have addressed that particular lacuna by adding a new chapter on statistical approaches to studying the biology and ecology of natural enemies. We hope that this will be useful to natural enemy biologists and, as it is written quite generally, also to researchers beyond our focal field.

We would like to thank all the contributors to this book for their sterling efforts in helping to bring this to fruition. Every previous chapter has been actively revised and updated since the second edition and has acquired at least one new co-author. It has been a privilege to work with both the 'old guard' and the relative newcomers, and with everyone else in between. They have all proven to be excellent natural enemy scientists. We also need to mention, with sadness, that Simon Leather, co-author of the substantial chapter on Populations and Communities, passed away before this book could be submitted for publication, although we are pleased to note that his own chapter was fully revised and completed before his demise.

The final phase of the preparation of this book was greatly assisted by us being in the same place at the same time for five months during the spring of 2022. We were among a group of Fellows at the Israel Institute for Advanced Studies (IIAS) studying 'Mathematical modeling of biological control interactions to support agriculture and conservation', as were a number of other chapter authors. We are deeply grateful to the IIAS for supporting this

research group programme and we also thank our co-Fellows for their interest in, and support of, our work on this edition.

We thank Bernie Roitberg for the cover photograph and George Heimpel for the photograph of Mark. We would, of course, also like to thank our colleagues at Springer, especially Zuzana Bernhart, for all their help during the (long) gestation period of the current edition.

We have tried to input approximately equally into the editorial process: we refer to this as the "Hardy–Wajnberg Equilibrium", and, indeed, we have waited many years for an opportunity to make that comment in print. We thank each other for our friendly but robust discussions and the associated détente. Having completed this third edition, we doubt that we ourselves will be the editors of a fourth, but we hope that in due course there will be further editions: this book has plenty of potential to assist parasitoid and predator researchers for years to come, especially if periodically updated to reflect new concepts, new techniques and new results. Our notion is that *Jervis's Natural Enemies* will serve as something akin to *Gray's Anatomy* (Standring, 2020) or, closer to our entomological homes, *The Insects* (Chapman, 2013). These books roll on through time, absorbing and disseminating the current state of the art. We therefore suggest that some of the younger researchers reading this book might, in due course, consider offering to construct a further edition. All in all, we hope to have borrowed and continued Mark's legacy in a manner, and to a standard, that would have pleased him and, as in the words of Scott Cook (see below), we hope now also to pass it along.

We hope that *Jervis's Insects as Natural Enemies: Practical Perspectives* will successfully both encourage and assist research into the fascinating biology of insect predation and parasitism.

Pass it along (excerpt)

Oh, and everywhere there are teachers, though some fell along the way.
The words they said still reach us, just like you're reaching me here today.
And you may not speak it loud, but it's clear in what you do.
And I hope to make you proud, 'cause I borrowed it from you'

Pass it along, pass it along. May it land in careful hands when we're gone.
You carry it for a moment but time won't loan it to you for long.

You don't own it, pass it along.
Scott Cook, One more time around (2013)

Helsinki, Finland — Ian C. W. Hardy
Sophia Antipolis, France — Eric Wajnberg
October 2022

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Standring, S. (2020) (ed) *Gray's Anatomy: The Anatomical Basis of Clinical Practice* (42nd edn). Elsevier, Amsterdam.

Preface to the Second Edition

The past three decades have seen a dramatic increase in practical and theoretical studies on insect natural enemies. The importance and appeal of insect predators, and of parasitoids in particular, as research animals derives from the relative ease with which many species can be cultured and experimented with in the laboratory, the simple life-cycles of most parasitoids, and the increasing demand for biological control of insect pests.

This book – an updated and considerably expanded version of Jervis and Kidd (1996) – is intended to guide enquiring students and research workers to those approaches and techniques that are most appropriate to the study and evaluation of predators and parasitoids. It is neither a practical manual nor a 'recipe book' – most chapters are accounts of major aspects of the biology of natural enemies, punctuated with advice on which experiments or observations to conduct and how, in broad terms, to carry them out. Detailed protocols are usually not given, but guidance is provided, where necessary, on literature that may need to be consulted on particular topics.

I hope that *Insects as Natural Enemies: A Practical Perspective* will successfully both encourage and assist research into the fascinating biology of insect predation and parasitism.

Cardiff, 2005 Mark A. Jervis

Reference

Jervis, M., & Kidd, N. (1996) (eds) *Insect Natural Enemies: Practical Approaches to Their Study and Evaluation*. Chapman and Hall, London, pp. 491.

Dedication: *For Julia, George and William, and in memory of my parents.*

Preface to the First Edition

The past two decades have seen a dramatic increase in practical and theoretical studies on insect natural enemies. The importance and appeal of insect predators, and of parasitoids in particular, as research animals derives from the relative ease with which many species can be cultured and experimented with in the laboratory, the simple life cycles of most parasitoids, and the increasing demand for biological control.

Unfortunately, despite a burgeoning of the literature on insect natural enemies, there has as yet been no general text available to guide enquiring students or research workers to those approaches and techniques that are most appropriate to the study and evaluation of such insects. Guidance on experimental design is particularly sought by newcomers to the subject together with some idea of the pitfalls associated with various approaches.

The need for such a book as this was realized as a result of our experiences in supervising students in the large entomology post-graduate school at the University of Wales, Cardiff. We also took our inspiration from *Aphid Technology*, edited by H.F. van Emden (1972), which satisfied an important need among entomologists and ecologists by providing practical advice on how to study a particular group of insects. Our book is aimed at any student or professional interested in investigating the biology of predators and parasitoids, but will, we hope, be especially useful to post-graduates.

Our book is neither a practical manual nor a recipe book. Most chapters are accounts of major aspects of the biology of natural enemies, punctuated by practical information and advice on which experiments or observations to conduct and how, in broad terms, to carry them out. Detailed protocols are usually not given. Guidance is also provided, where necessary, on literature that may need to be consulted on particular topics. The coverage of the book is far from being exhaustive; some readers may be surprised at the omission of important topics such as dormancy, and others may be appalled that there is negligible treatment of systematics! For this we apologise, but space was at a premium. Statistical aspects of sampling and experimental design are hardly gone into. This may be seen by some as an unforgivable lapse, but a wealth of study is already contained in texts such as Southwood (1978), McDonald et al. (1989), Mead (1989), Hairston (1989) and Crawley (1993) (the latter text is concerned with a particularly valuable software tool, GLIM). Ecologically minded students of natural enemies will also find valuable advice in the special feature on statistics published in the September 1993 issue of the journal *Ecology*.

We hope that *Insect Natural Enemies* will successfully both encourage and assist the reader in his or her research into insect predators and parasitoids.

Cardiff, 1996

Mark A. Jervis
Neil A.C. Kidd

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Foraging Behaviour

1

Mark D. E. Fellowes, Jacques J. M. van Alphen, K. S. Shameer, Ian C. W. Hardy, Eric Wajnberg, and Mark A. Jervis

1.1 Behaviour of Insect Parasitoids and Predators

In this chapter, we consider practical aspects of the foraging behaviour of insect natural enemies in its widest sense (so wide that we even include a few examples concerning non-insect arthopods, such as mites). Initially, most insect natural enemies must locate the habitat where potential victims may be found. Within that habitat, the victims themselves must be discovered. Once a patch of potential targets is identified, the predator or female parasitoid must choose its victim. Furthermore, in judging host quality, a female parasitoid must decide whether to feed from the host, to oviposit, or to do both. If she does decide to oviposit, then there are questions of sex allocation and offspring number that need to be addressed (Fig. 1.1). All of these activities fall under the aegis of 'foraging behaviour'.

Studies of the foraging behaviour of insect natural enemies lie at the heart of much of modern ecology. These studies have taken two broadly defined pathways, where the emphasis is determined by the interest of the researcher (see below). Irrespective of the motivation of the researcher, it is clear that any attempt to understand the foraging behaviour of a predator or a parasitoid will greatly benefit from knowledge gleaned from both approaches. This cross-fertilisation of ideas is something we try to emphasise in this chapter.

In addition, we provide a review of the foraging behaviour of insect natural enemies. This is meant to be illustrative, with stress placed on the experiments used to study the behaviour itself. For greater detail on the behaviour of parasitoids one should refer to Godfray (1994), Quicke

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Fig. 1.1 Foraging decisions. In adopting either the functional or the causal approach to studying predator and parasitoid behaviour, it is useful to consider that foraging insect natural enemies are faced with a number of consecutive or simultaneous decisions. Listed in this figure are some of the questions that may need to be addressed by a gregarious host-feeding parasitoid

(1997) and Wajnberg et al. (2008) and, for a shorter overview, to Hardy and Godfray (2023). The literature on insect predators is much more diffuse, but New (1991) provides a good introduction to the behaviour of predators in general, while Dixon (2000) reviews the behaviour of ladybird beetles (Coccinellidae), and Davies et al. (2012) provide an excellent introduction to many aspects of animal behaviour in general.

Where possible, we deal with insect predators and parasitoids together, although there are some sections (e.g., sex allocation, Sect. 1.11) where the examples come exclusively from the parasitoid literature, and other sections (e.g., superparasitism/cannibalism, Sect. 1.9) where both are dealt with, but separately. Nevertheless, many of the approaches to studying foraging behaviour, and the theory underpinning it, are similar for both predators and parasitoids.

In this chapter, we first describe the methodological approaches that underpin studies of the foraging behaviour of insect natural enemies. Second, we discuss how the predators and parasitoids find the habitat patches where potential prey or hosts may be encountered. Third, we reflect on what occurs after the prey or host is found, dealing with issues such as clutch size and sex allocation decisions, and patch defence behaviour. We also deal with considerations such as the cost of reproduction to natural enemies and the resistance of their hosts or prey to being exploited. Finally, we touch briefly upon some of the wider population and ecological consequences of insect natural enemy foraging behaviour.

1.2 Methodology

1.2.1 The Causal Approach

Until the late 1970s, parasitoid foraging behaviour was mostly studied from a proximate (i.e., causal or mechanistic) standpoint, with a strong emphasis on identifying which stimuli parasitoids respond to both in finding and in recognising their hosts. Through this approach, fascinating insights into parasitoid foraging behaviour have been gained, and it has been demonstrated that often an intricate tritrophic relationship exists between phytophagous insects, their host plants and parasitoids. We now know the identities of some of the chemical compounds eliciting certain behaviours in parasitoids. Some of the research in this field has been devoted to the application to crops of chemical substances, as a mean of manipulating parasitoid behaviour in such a way that parasitism of crop pests is increased. Many parasitoid

species display individual plasticity in their responses to different cues. Associative learning (Sect. 1.6.2) of odours, colours or shapes related to the host's environment has been described for many parasitoid species (e.g., Dugatkin & Alfieri, 2003; Meiners et al., 2003a).

Often, causal questions do not involve elaborate theories. Questions of whether an organism responds to a particular chemical stimulus or not, or whether it reacts more strongly to one stimulus than to another, lead to straightforward experimental designs. It is in the technical aspects of the experiment rather than the underlying theory that the experimenter needs to be creative. However, the study of causation can be extended to ask how information is processed by the central nervous system. One can ask how a sequence of different stimuli influences the behavioural response of the animal, or how responses to the same cue may vary depending on previous experience and the internal state of the animal (Putters & van den Assem, 1988; Morris & Fellowes, 2002).

Two different causal approaches have been adopted in the study of the integrated action of a series of different stimuli on the behaviour of a foraging animal:

1. The formalisation of a hypothesis into a model of how both external information and the internal state of the animal result in behaviour, and the testing, through experiments, of the predictions of the model. Waage (1979) pioneered this approach for parasitoids. Artificial neural network models have also been used to analyse sex allocation behaviour in parasitoids (Putters & Vonk, 1991; Vonk et al., 1991).
2. The statistical analysis of time-series of behaviour to assess how the timing and sequence of events influences the behaviour of the organism. An example of this approach is the analysis of the factors influencing patch time allocation of a parasitoid, using the proportional hazards model (Haccou et al., 1991; Wajnberg et al., 1999; Tenhumberg et al., 2001a; Wajnberg, 2003, 2004, 2006 Burger et al., 2006; Parent et al., 2017).

1.2.2 The Functional Approach

The functional approach to the study of parasitoid behaviour is based on Darwinian ideas initially formalised by MacArthur and Pianka (1966) and Emlen (1966). Termed 'natural selection thinking' by Charnov (1982), it asks how natural selection may have moulded the behaviour under study.

Because foraging decisions (Fig. 1.1) determine the number of offspring produced, foraging behaviour must be under strong selection pressures. Assuming that natural selection has shaped parasitoid searching and oviposition behaviour in such a way that it maximises the probability of leaving as many healthy offspring as possible, thus maximising the ability to contribute genetically to the next generations, it is possible to predict the optimised behaviour under given circumstances. In the real world, no 'Darwinian monsters' exist that can produce limitless numbers of offspring at zero cost. Because resources are often limited and because reproduction incurs a cost (e.g., in materials and energy and foraging time, Chap. 2) to an individual, increasing investment in reproduction must always be traded off against other factors decreasing fitness (e.g., more offspring often means smaller individual offspring with shorter lifespans or lower competitive abilities). Thus, producing the maximum possible number of offspring may not be the optimal strategy.

We refer to natural selection thinking as the functional approach, because its aim is to define the function of a particular behaviour. To achieve this goal, it is necessary to show that the behaviour contributes more to the animal's fitness than alternative behaviours in the same situations. The foraging behaviour of female parasitoids has a direct influence on both the number and the quality of their offspring, so it is particularly suited for testing optimisation hypotheses. The functional approach can be applied not only to theoretical problems but also to problems such as the selection, the evaluation and the mass rearing of natural enemies and their efficacy in

biological control (van Lenteren, 2003; Plouvier & Wajnberg, 2018; Chap. 7).

There are several (related) ways of investigating functional problems in behavioural ecology, all using quantitative optimality models. One is to predict the optimised behaviour under given and relatively fixed environmental conditions, considering that the state of the foraging animal (e.g., its egg load, age, energy reserve, etc.) remains fixed throughout its lifetime. These models may be referred to as 'static'. Other approaches explicitly take into account that the state of the animal can change, essentially due to its foraging activity and its success in finding and exploiting resources. This class of models may be referred to as 'dynamic'. Usually, for these two types of model, the environment does not contain other competing decision-makers. In this chapter, these two first classes of model will be referred to as 'classical models'. A third class of models takes into account the possibility that the optimal behavioural strategy will be dependent on what other individuals, attacking the same host or prey population (i.e., competing decision-makers), are doing, since these competitors are also trying to optimise their own foraging decisions. This third class of model is based on game theory. All approaches can be used to inform practical studies of insect natural enemies, and in a similar fashion, the results of practical studies can be used to construct more realistic models.

Classical Optimality Models

Optimality models are used to predict how an animal should behave so as to maximise its fitness in the long term. Classical optimality models do not explicitly take into account the foraging decisions taken by competing decision-makers. They can be designed by determining:

1. What decision assumptions apply, i.e., which of the forager's choices (problems) are to be analysed. Some of the decisions faced by foraging natural enemies are shown in Fig. 1.1. Sexually reproducing gregarious parasitoids need to make the simultaneous decision not only of what size of clutch to lay but also of what sex ratio of progeny to produce. The progeny and sex allocation of such parasitoids may be easier to model if the two components are assessed independently; i.e., it is assumed that the female need make only one decision. In a formal model, the decision studied must be expressed as one or more algebraic decision variables (see, e.g., Wajnberg, 2012). In some models of progeny (clutch size) allocation, the decision variable is the number of eggs laid per host, while in most models of patch exploitation the decision variable is patch residence time.
2. What currency assumptions or optimality criteria apply, i.e., how the various choices are to be evaluated. A model's currency is the criterion used to compare alternative values of the decision variable (in other words, it is what is taken to be maximised by the animal in the short term for long-term fitness gain). For example, some foraging models maximise the net rate of energy gain per time unit while foraging, whereas others maximise the fitness of offspring per host attacked.
3. What constraint assumptions apply, i.e., what factors limit the animal's choices, and what limits the 'payoff' that may be obtained. There may be various types of constraint upon foragers. These range from the phylogenetic, through the developmental, physiological and behavioural, to the animal's time budget. Taking as an example clutch size in parasitoids, and the constraints there may be on a female's behavioural options, an obvious constraint is the female's lifetime pattern of egg production. In a species that develops eggs continuously throughout its life, the optimal clutch size may be larger than the number of eggs a female can possibly produce at any one time. An example of both a behavioural and a time-budget constraint upon the behavioural options of both parasitoids and predators is the inability of the forager to handle and search for prey simultaneously. Here, time spent handling the prey

is at the cost of searching for further prey. For a detailed discussion of the elements of foraging models, see Stephens and Krebs (1986) or Cézilly and Benhamou (1996).

Sometimes the investigator knows, either from the existing literature or from personal experience, the best choices of decision assumption, currency assumption or constraint assumption. If it is impossible to decide on these based on existing knowledge, one can build models for each alternative and compare the predictions of each model with the observed behaviour of the parasitoid or predator. In this way, it is possible to gain insight into the nature of the selective forces working on the insect under study (Waage & Godfray, 1985; Mangel, 1989; Cézilly & Benhamou, 1996).

Classical optimality models assume a static world in which individual parasitoids search for hosts. While these models are still useful research tools, they ignored the possibility that for a forager, today's decision may affect tomorrow's internal state, which may in turn affect tomorrow's decision, and so on. The internal state of a searching parasitoid changes during adult life: its egg load (the number of mature eggs in the ovaries) and its energy reserves may decrease, and the probability that it will survive to another day decreases. The optimal behavioural strategy will depend on these changes. Likewise, the environment is not static. Bad weather or the start of an unfavourable season can also influence the optimal strategy. Dynamic foraging models have been subsequently designed to take into account internal physiological changes and changes in the environment (Mangel & Clark, 1988; Chan & Godfray, 1993; Weisser & Houston, 1993, Tenhumberg et al., 2001b).

Implicit in some optimality models is the assumption that the forager is omniscient or capable of calculation, e.g., that a parasitoid wasp has some knowledge of the relative profitability of different patches without actually visiting them (Cook & Hubbard, 1977). Behavioural studies on parasitoids have shown, however, that insects can behave optimally by employing very simple quick 'rule' mechanisms such as the mechanism determining patch time allocation in *Venturia canescens* described in Sect. 1.5 and the males-first mechanism used by some species in progeny sex allocation (Sect. 1.11.5 and Fig. 1.19). These mechanisms approximate well the optimal solution in each case.

Evolutionarily Stable Strategies

Almost all parasitoids leave the host in situ. Thus, there is always the possibility that other parasitoids may find the same host and also oviposit in it. The optimal behaviour of the first female thus depends on what other parasitoids may do (i.e., the environment of a focal individual contains other competing individuals), especially since other individuals are also expected to to adopt their own optimal reproductive behaviours. Likewise, the best time allocation strategy for a parasitoid leaving a patch in which it has parasitised a number of hosts depends both on the probability that other wasps will visit that patch and on the probability that other parasitoids may have already exploited the patches it visits next. For this reason, problems concerning the allocation of patch time, progeny and sex require models in which the evolutionarily stable strategy (ESS; Maynard Smith, 1974; Mesterton-Gibbons, 2019) is calculated. The ESS approach asks what will happen in a population of individuals that play all possible alternative strategies, and is based on game theory. The fact that individuals lack control over all decision variables affecting their rewards is what makes such situations a game, and what distinguishes them from classical optimisation problems (Mesterton-Gibbons, 2019). A strategy is an ESS if, when adopted by most members of a population, it cannot be invaded by the spread of any rare alternative strategy (Maynard Smith, 1972). In seeking an ESS, theoreticians are looking for a strategy that is robust against mutants playing alternative

strategies. The ESS, like the optimum in models for single individuals, is calculated using a cost-benefit analysis. We refer the reader to Maynard Smith (1982), Parker (1984) and Mesterton-Gibbons (2019) for descriptions of well-explored ESS models, details of how to calculate the ESS and their use in behavioural ecology, and to Hardy and Mesterton-Gibbons (2023) for a recent discussion of game theory in relation to natural enemy behaviour.

Why Use Classical Optimality and ESS Models?

Sometimes, experimental tests of optimality and ESS models will produce results not predicted by the models. At other times, only some of the predictions of the theoretical model are confirmed by empirical tests. Rarely is a perfect quantitative fit between model predictions and empirical test results obtained. Irrespective of whether a good fit is obtained, valuable insights are likely to be gained into the behaviour of the insect. Construction of models helps in the precise formulation of hypotheses and quantitative predictions and allows us to formulate new hypotheses when the predictions of our model are not met. Thus, classical optimality and ESS models are nothing more or less than research tools.

Ideally, both causal and functional questions should be asked when studying the foraging behaviour of insect parasitoids and predators. In the sections on superparasitism (Sect. 1.9.4) and patch time allocation (Sect. 1.5), we will show how, by ignoring functional questions, one may hamper the interpretation of data gathered to establish that a certain mechanism is responsible for some type of behaviour. Ignoring causal questions can likewise hamper research aimed at elucidating the function of a behavioural pattern; e.g., research into causal factors can demonstrate the existence of a constraint, not accounted for in a functional model, upon the behaviour of the parasitoid. Both causal and functional approaches are required for a thorough understanding of parasitoid behaviour.

1.2.3 The Comparative Method

Introduction

Perhaps the approach with the longest pedigree in studying animal behaviour is the comparative method. With this method, data are collated across species, and a search is made for statistical patterns (Harvey & Pagel, 1991). One advantage of this method is that data are often already available (although often widely scattered) in the literature (e.g., see analyses by Blackburn, 1991a, 1991b; Mayhew & Blackburn, 1999; Jervis et al., 2001, 2003). Until recently, sets of species average data were usually analysed in much the same way as within-species data. However, the fundamental assumption of most early statistical analyses—that species-comparative data are independent observations (i.e., are independent of each other)—may not hold. Also, we want to know whether observed interspecific similarities have evolutionary meanings in the present time or whether they derive from common ancestors in the phylogenetic tree. Cross-species data may actually be non-independent, because the species are related through phylogeny (i.e., they share an evolutionary history). Comparative biologists have developed methods which use phylogenetic information in conjunction with species data sets to generate independent values for statistical analysis and to enable a more accurate evolutionary interpretation of interspecific observed similarities or differences (Felsenstein, 1985; Harvey & Pagel, 1991; Harvey & Nee, 1997; Freckleton et al., 2002; Wajnberg et al., 2003).

The Method of Independent Contrasts

Probably the most commonly employed method involves ‘independent comparisons’, also known as ‘independent contrasts’ (originally developed by Felsenstein, 1985: simple examples are given in Harvey & Pagel, 1991; Purvis & Rambaut, 1995; Harvey, 1996; Mayhew & Pen, 2002). The approach assumes that the branches of a phylogeny can be modelled by a Brownian motion

process, such that successive changes are independent of one another and that the expected total change summed over many independent changes is zero. The original method for contrast analysis assumes that the lengths of branches in the phylogeny are known, but often they are not, in which case they can be assumed to be equal (e.g., Jervis et al., 2001, 2003). Branch lengths are estimated by genetic distances (divergence times, in relation to the present, estimated from the fossil record or from molecular clocks) or by the number of character changes, determined from a cladistic analysis (Harvey & Pagel, 1991).

An independent contrast is obtained from each node in the phylogeny for each measured variable. Imagine that you are studying five species (Fig. 1.2a): it is clear that A and B, and X and Y are more closely related to each other than to members of the other clade, and, by comparing their values, we then only include independent evolutionary trajectories. By calculating an ancestral trait value at the node below A-B and X-Y, we gain another contrast. By comparing this ancestral node value with the value for species Z, we gain another independent contrast. Therefore, we gain four contrasts from five species. If two traits are of interest in the comparative analysis and if they are continuous variables, then typically the data are analysed using a linear regression, constrained to pass through the origin (i.e., there should be no intercept in the regression model, Garland et al., 1992).

As we have seen, with a perfectly resolved phylogeny of n species, there are $n - 1$ possible contrasts available. This may result in statistical difficulties when data sets are small. While this problem may be alleviated by the addition of

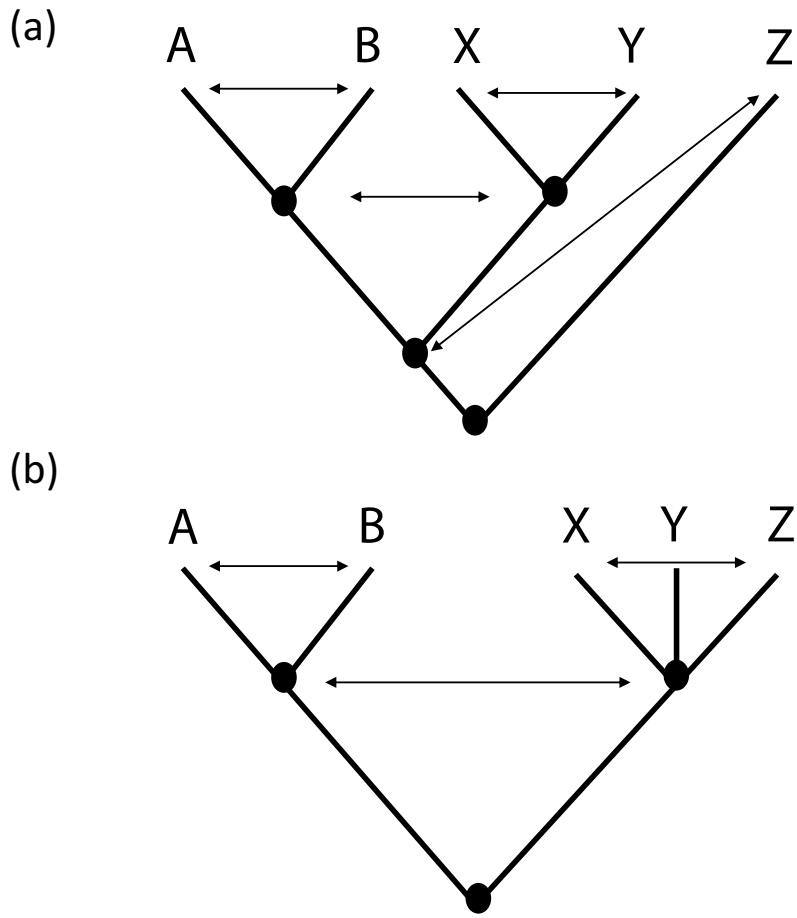


Fig. 1.2 Independent contrasts: For n species, there are $(n - 1)$ independent contrasts which can be calculated for a fully phylogeny that is fully resolved at each node (**a**). Where the phylogeny is less well resolved, resulting in polytomies at some nodes, the number of possible independent contrasts is diminished (**b**), reducing the potential power of the analysis.Contrasts are shown by double headed arrows, nodes by dots and the species considered are A, B, X, Y and Z

extra species to the analysis, a more invidious difficulty is introduced when a phylogeny is poorly resolved. While phylogenies usually consist of bifurcating lineages, for some taxa the phylogeny may not be well resolved, and so it will contain polytomies (trifurcations, etc.) and hence fewer nodes for a given number of species. Thus, the number of contrasts obtained is less than with a fully resolved (bifurcating) tree, reducing the size of the data set and also the statistical power of subsequent analyses. Reconsider the figure with species A, B, X, Y and Z (Fig. 1.2a). If we do not know the evolutionary relationships among X, Y and Z, we must assume that they all originated from the same common ancestor (a polytomy; Fig. 1.2b). We now have fewer contrasts and hence lower statistical power in the analysis. Such problems can be overcome by obtaining a sufficiently well-resolved phylogeny. Unfortunately, such phylogenies are not always available. Garland and Díaz-Uriate (1999) provide further discussion.

Although a well-resolved, published taxonomy (more often than not, based exclusively on morphology) can be used to approximate the true phylogenetic tree (e.g., Abram et al., 2023), it is important to be aware that some currently accepted taxonomic groupings may not be monophyletic, i.e., they may not contain all the descendants of a common ancestor. Phylogeny-based comparative methods assume groupings to be monophyletic, so using an incorrect phylogeny will seriously undermine the value of the analyses undertaken. In the absence of molecular-based (i.e., DNA) phylogenies, cladistically-based taxonomies are the most suitable taxonomies for comparative studies as they are intended to closely reflect phylogeny.

There are now a number of software packages available that allow users to perform rigorous comparative analyses, many of which are freely available over the internet (http://evolution.genetics.washington.edu/phylip/software.html provides access to many of these packages, and more). Several R packages (R Core Team, 2020) are also available for that, e.g., caper (Orme, 2012), ape (Paradis et al., 2004), geiger (Harmon, 2009), etc.

The comparative method can also be used to make predictions concerning the ecology of a species. Hardy et al. (1992a), used a phylogenetic tree, based solely on morphological characters (now published incorporating molecular data; Schilthuizen et al., 1998), of the six *Drosophila* parasitoid species of *Leptopilina* occurring in Europe, to predict where in the environment *L. longipes*, a species whose hosts and host habitat were unknown, would be found (Fig. 1.3). The five other species are all parasitoids of *Drosophila*. The tree divides initially into two branches. When examining how the character 'host habitat choice' is distributed over the tree (i.e., the character is 'mapped' onto the tree), it appears that the upper branch of the tree contains the species finding its hosts in fermenting fruits (*L. heterotoma*), while the other branch contains species finding their hosts in fungi and/or decaying plant matter (*L. clavipes*, *L. australis* and *L. fimbriata*). Because *L. longipes* is most closely related to *L. fimbriata*, it was predicted that it is attracted, like its close relative, to decaying plant material. Subsequently, *L. longipes* was trapped with baits comprising rotting cucumber containing *Drosophila* larvae, and during fieldwork it was also found on decaying stalks of the umbellifer *Heracleum* and on fungi.

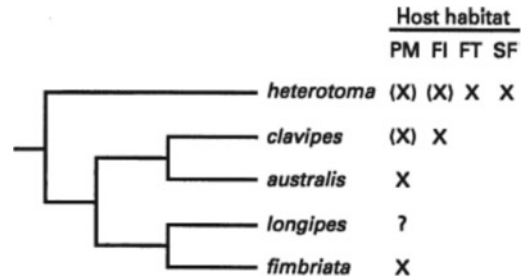


Fig. 1.3 Cladogram, based on adult morphology, of the *Leptopilina* species (Hymenoptera: Eucoilidae, parasitoids of *Drosophila*) occurring in northwestern Europe. Microhabitat use is 'mapped' onto the ends of the tree branches. X = principal microhabitat, (X) = microhabitat from which a species has occasionally been recovered. PM = decaying plant material; FI = fungi; FT = fermenting fruit; SF = sap fluxes. Microhabitat use by *L. longipes* was predicted from that species' position on the cladogram

1.3 The Treatment of Parasitoids Prior to Their Use in Experiments

1.3.1 Rearing

The species and quality of the host a parasitoid is reared on can have a marked influence on its subsequent behaviour, for example through its effect on egg load and life expectancy (Sects. 2.7.3 and 2.8.3). While the influence of the host-related phenotypic variation in natural enemy traits on the results of laboratory trials may generally be mimimised or even avoided by altering the rearing regime, more insidious problems arise when insect populations are reared in mass culture. The first problem is ubiquitous and unavoidable. Natural selection will operate in the controlled environment room as much as anywhere else, changing the genetic composition of the population and, as a result, potentially influencing the behaviour of the population of interest (e.g., Matos et al., 2000; Simoes et al., 2007; Burke & Rose, 2009; Diamantidis et al., 2011; Hoffmann & Ross, 2018). There are two approaches to avoiding the complications of such adaptation. The first is to simply measure the traits of interest before significant selection occurs, so ideally few generations will have passed between capture and experiment. Second, if one is interested in using selection experiments to probe the nature of the trait, then we recommended that outbred populations of the species of interest be maintained for at least ten generations in the laboratory. This allows adaptation to laboratory conditions to occur and should avoid complications from any inadvertent selection pressures during the experiment. Further, maintenance of separate lines from an initially common population can also be used as a tool to generate and then test the effects of genetic differences (Mathiron et al., 2019).

A more serious problem results from small effective population sizes, leading to genetic drift and inbreeding depression. Testing the variation in an inbred population, at best, results in an underestimate of the variation in natural populations, and, at worst, provides a skewed view of the true variation present.

1.3.2 Experience

It has been shown for several parasitoid species that an individual's previous experience can modify its behaviour (Sect. 1.6.2). This phenomenon has been observed in all phases of the foraging process and often involves responses to chemical stimuli (Vet & Dicke, 1992). For instance, females *Goniozus nephantidis*, a gregarious ectoparasitoid naturally associated with *Opisina arenosella*, that have developed on the factitious host *Corcyra cephalonica*, prefer *C. cephalonica* when offered a choice between the two host species, but prefer *O. arenosella* after having been exposed to their odour (Subaharan et al., 2005). Previous ovipositions in hosts of a certain species can also influence host species selection in choice experiments (van Alphen & Vet, 1986), while the decision to oviposit into an already parasitised host (i.e., superparasitism) also depends on previous experience with unparasitised or parasitised hosts (Visser et al., 1992b; Hubbard et al., 1999; Chen et al., 2020; Ayala et al., 2021). Thus, when designing experiments, one should always be aware that the previous history of an individual may influence its behaviour (as may the ecological history of the population the individual is drawn from, Vyas et al., 2019). Such history can affect the results of experiments on patch time allocation, superparasitism and also the results of experiments in which interactions between adult parasitoids are studied. Storing parasitoids in the absence of either hosts or host-related cues can have an effect. Visser et al. (1990) showed that it matters whether wasps are stored in a vial singly or with other females prior to conducting an experiment. Such effects have also caused problems when parasitoids and hosts are mass reared for biological control purposes. Rearing the apple pest *Cydia pomonella* on an artificial diet reduced the ability of the parasitoid *Hyssopus*

pallidus to respond to host location cues, as it changed the composition of the kairomones normally found in the host's frass (Gandolfi et al., 2003).

Conditioning parasitoids, by allowing them to search and oviposit for some time before an experiment, can nonetheless be a sensible practice. Inexperienced parasitoids often show lower encounter rates and are less successful in handling their hosts (Samson-Boshuizen et al., 1974). By allowing parasitoids access to hosts before they are actually used in an experiment, one can often save many hours that would otherwise be wasted in observing parasitoids that are 'unwilling' to search. Often, however, it is advisable to use freshly emerged, inexperienced females, for example in choice experiments, either where different host plants, host instars or host species are offered or where the olfactory responses of parasitoids to different chemicals are studied.

Often, one is interested in the performance of natural populations. These comprise individuals with different experiences and/or different degrees of experience, so using only inexperienced females in the laboratory gives a distorted view of what happens in nature. One approach is to collect adults from the field for study in the laboratory. A large enough sample should give a reasonable idea of how individuals in the population behave on average. However, one should be aware of the problems of genotype-by-environment interactions, where not all genotypes respond to changes in environment in the same way. Ideally, the laboratory conditions will reflect what is likely to be encountered in the field, especially in terms of temperature.

Because experience can influence subsequent behaviour, the results of experiments in which an insect encounters two situations in succession can depend on which situation is encountered first. In such cases, one should take care that in half of the replicates one situation is encountered first, while in the other half the sequence is reversed.

1.3.3 Sex Ratio

While such effects of experience can have a great influence on the behaviour of insects, more subtle problems ought to be borne in mind. An often overlooked problem in sex ratio studies is the possible presence of *Wolbachia* and other male-killing bacteria in the study organism (Ode & Hardy, 2008; Chaps. 3, 5 and 6). For example, Majerus et al. (1998) found that almost 50% of females from a Japanese population of the coccinellid *Harmonia axyridis* were attacked by a male-killing bacterium, resulting in a heavily female-biased sex ratio. Those ladybirds from a Mongolian and a Russian population had low (<2%) or no infection. To confirm the presence of bacteria, one can simply 'cure' the experimental individuals by treating them with antibiotics (Sects. 3.4.2 and 6.5).

Selfish genetic elements (regions of the chromosome that are inherited in a non-Mendelian manner during segregation, resulting in their becoming over-represented in gametes) provide another means of sex ratio distortion (Ode & Hardy, 2008; Chap. 3). *Nasonia vitripennis* has been found to commonly carry *psr* (parental sex ratio), a selfish genetic element that results in the production of male-only broods by causing fertilised eggs (normally female) to become male. Such distortion of the sex ratio will have a considerable influence on the population ecology of *N. vitripennis* (reviewed in Godfray, 1994; see also Chap. 5) and could potentially influence the outcome of sex ratio studies if present in a laboratory culture.

More often, changes in sex ratios will result from conditional sex allocation (Sect. 1.11.3) or local mate competition (Sect. 1.11.2). Bernal et al. (1999) found that two species of *Metaphycus*, parasitoids of scale insects, showed much more female-biased sex ratios if provided with larger hosts. This is likely to result from conditional sex allocation (where female parasitoids preferentially place female offspring in larger hosts). Since these parasitoids may be used

as biocontrol agents attacking scale insect pests of citrus trees, using rearing protocols that maximise the proportion of females would be economically sensible (Bernal et al., 1999; Ode & Heinz, 2002; Chow & Heinz, 2005; reviewed in Ode & Hardy, 2008).

1.4 Handling Behavioural Data

1.4.1 Recording Behaviour

The equipment used to record insect behaviour has developed rapidly, driven primarily by advances in computing power. Nevertheless, many (if not most) studies of insect foraging behaviour rely upon direct observation and note-taking. This approach is not without drawbacks, in that it is difficult to avoid bias in recording. The simplest way around this is to use video-recording equipment, so that two independent observers can time and assess the behaviours of interest. A development of such techniques involves 'intelligent' video systems, which have a number of advantages (Chap. 4).

1.4.2 Analysing Behavioural Data

Because insects may change their behaviour in response to experiences gained while foraging, and because their internal state (e.g., egg load) changes during the foraging process, the different behavioural events of the same individual during an observation period are not independent. The standard statistical methods described in numerous textbooks are in general inappropriate for the analysis of some behavioural data because they do not adequately take into account the connection between the succession as well as the duration of acts. Haccou and Meelis' (1992) book on the statistical analysis of behavioural events is recommended as a useful introduction to the most appropriate approach (see also Wajnberg & Haccou, 2008, for additional information).

1.4.3 Behavioural Research in the Field

Whether behavioural research is aimed at answering fundamental questions or deals with the use of parasitoids and predators in biological control, the ultimate goal of interest is the performance of the insects in the field (Heimpel & Casas, 2008). The small size of many parasitoids makes observation of their behaviour in the field often difficult or impossible. This applies particularly to the monitoring of the movements of individuals, for example between patches. Following Hassell and Southwood (1978), patches can be defined either as units of host or prey spatial distribution or as limited areas in which natural enemies search for hosts or prey; often there is a hierarchy of patches, e.g., tree, branch, leaf, leaf-mine. The movements of larger insects, such as ichneumonids and sphecids, can be more easily observed. Dispersal of small parasitoids in the field can be studied by placing patches with hosts (e.g., potted, host-bearing plants) and releasing marked adults. By checking the host plants at regular intervals for the presence of marked individuals, it is possible to obtain information on the speed at which the insects move between host plants, on the time they spend searching each patch and on the spatial distribution of parasitoids over the available patches. When hosts are later examined, the aforementioned data can be related to the amount of parasitism in each patch.

By using marked parasitoid individuals, one can distinguish between insects released for the experiment and those occurring naturally. Large wasps can be marked with paint on the thorax, using a fine paintbrush (acrylic paint was used by, e.g., Driessen & Hemerik, 1992; Petersen & Hardy, 1996; Snart et al., 2018). By using different colours or colour combinations one can distinguish between different individuals, or groups. Small wasps can be marked with fluorescent dusts, but this has the disadvantage that one may need to remove wasps from the

experimental plot to detect the dust mark under ultraviolet light. For some species, it may be useful to mark indivuals by rearing them on diet containing deuterium, which alters the chemicals that adults subsequently emit in detectable manner (Goubault & Hardy, 2007). Genetic markers (Chap. 3) have also been used to monitor parasitoids in the field (Kazmer & Luck, 1995). Other workers have suggested that phenotypically distinguishable mutants may prove useful in studying population dynamics, although there are obvious drawbacks with this approach (Snodgrass, 2002).

Many species, when observed in the field, continue foraging normally. Janssen (1989) used a stereomicroscope mounted on a tripod in the field to observe the foraging behaviour of parasitoids on patches (sap streams and fermenting fruits) containing *Drosophila* larvae. Casas (1990) also recorded the behaviour of *Sympiesis sericeicornis* while the parasitoid searched for its leafminer host on potted apple trees in the field. To characterise the relationship between egg loads and sugar availability in actively foraging parasitoids from the field, Segoli and Rosenheim (2013a, 2013b) collected *Anagrus daanei* and *A. erythroneurae* by shaking the grape canes above a white plastic cafeteria tray (25 × 36 cm) several times at each site during different seasons. The number of cane shakes depended on the number of parasitoids falling on the trays because of the time taken to put them in vials, this also allowed them to estimate the number of parasitoids captured per shake as a primary measure of parasitoid abundance. To study the relationship between oviposition success and body size of female parasitoids, modified collection trays with a system of baffles were used to collect minute parasitoid wasps (<1 mm) of *Anagrus sophiae* from the field, which forage on *Spartina* foliage for planthopper eggs and, upon death, fall out of the plant canopy (Segoli & Rosenheim, 2015). Other natural enemy species are easily disturbed when approached, and disturbance can be avoided in some cases by using binoculars (Waage, 1983).

1.5 Patch Time Allocation

1.5.1 Introduction

One aspect of parasitoid foraging behaviour where the causal approach and the functional approach have traditionally coexisted is patch time allocation. We will consider first which factors affect patch time allocation and second how one can analyse the interplay of the different factors.

1.5.2 Factors Affecting Patch Time Allocation

Patch time allocation in parasitic wasps is likely to be affected by the following (Wajnberg, 2006):

1. A parasitoid's previous experience;
2. Its internal state (e.g., egg load, energy reserves);
3. Patch kairomone concentration;
4. Encounters with unparasitised hosts;
5. Encounters with parasitised hosts;
6. The timing of encounters and attacks of healthy and already attacked hosts;
7. Whether the females lay a son or a daughter egg;
8. Encounters with the marks of other parasitoids;
9. Encounters with other parasitoid individuals;
10. Superparasitism;
11. Genetic variation.

Some of these factors can be studied through experiments in which all the other factors are excluded. For example, the effect of kairomone concentration can be investigated without involving hosts at all (Sect. 1.6). To eliminate the effects of encounters with other parasitoids and their marks, the experimental design shown in Fig. 1.4 can be used. However, it may be impossible with some experiments to separate the effects of different factors. A notorious problem is the analysis of the factors that determine how

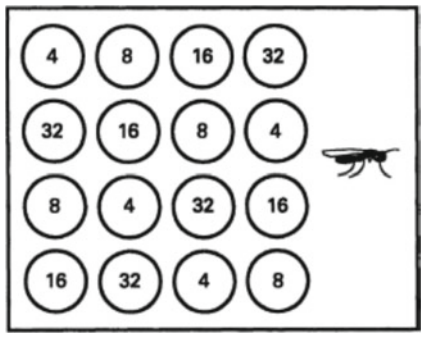


Fig. 1.4 Patch time allocation by individual parasitoids and predators. Schematic representation of one suggested experimental design for an experiment for studying patch time allocation. A randomised arrangement of patches (denoted by circles) is used. Numbers within circles indicate the number of hosts present in each patch. This experimental design can be used in the study of aggregative responses

long a parasitoid will stay on a patch that initially contains only unparasitised hosts. Because the parasitoid oviposits in the unparasitised hosts it encounters, the number of unparasitised hosts decreases while the number of parasitised hosts increases. Thus, with the passage of time, the parasitoid experiences a decreasing encounter rate with unparasitised hosts and an increasing encounter rate with parasitised hosts. Because both the temporal spacing and the sequence of encounters with parasitised and unparasitised hosts are stochastic in nature, encounter rates with both types of host do not alter in a monotonic, smooth fashion. However, modern statistical analysis tools can take into account such situations (see Wajnberg, 2006, for a review), and modelling approaches can also provide some help (see, e.g., Pierre et al., 2012).

In some parasitoid species, encounters with unparasitised hosts have an incremental effect on the time spent in a patch (van Alphen & Galis, 1983; Haccou et al., 1991). This poses the question: 'What effect do encounters with *parasitised* hosts have on patch time allocation, and how does the relative timing of encounters with parasitised and unparasitised hosts influence the period spent in individual patches?'.

1.5.3 Analysing the Interplay of Different Factors

Two distinct hypotheses can be formulated about the effect of encounters with healthy and parasitised hosts on patch residence times. The functional hypothesis is as follows: given that a parasitoid is able to discriminate between parasitised and unparasitised hosts, encounter rates with both host types provide the parasitoid with information on host density and the degree of exploitation of a patch. This information allows the wasp to determine when to leave the patch, e.g., high encounter rates with parasitised hosts in combination with low encounter rates with unparasitised hosts signal a high level of exploitation of the patch. Because it could be more profitable for the wasp to move on and search for a higher-quality patch, the insect might decide to leave. Van Lenteren (1976) recognised this as one of the functions of host discrimination and showed, through single-patch experiments, that wasps continued to search on patches in which parasitised hosts were immediately replaced by unparasitised ones, whereas wasps allowed to search on similar but unreplenished patches attempted to leave the experimental arena after most of the hosts had been parasitised. The functional hypothesis states that encounters with both unparasitised and parasitised hosts affect patch time, but it does not specify the mechanism involved.

The causal hypothesis formulates explicitly how encounters with healthy and parasitised hosts affect patch time. This hypothesis is an extension of a mechanistic model for patch time allocation proposed by Waage (1979) for the parasitoid *Venturia canescens*. Although this model was shown to be an incorrect description of the behaviour of *V. canescens* (Driessen et al., 1995, see also Pierre et al., 2012), it is still valuable as a conceptual model, and it can be applied to many other parasitoid species. Waage (1979) assumed that a female parasitoid, when entering a patch containing hosts, has a certain motivation level for searching the patch, the level

being set by previous experience and kairomone concentration on the patch. If the wasp does not locate and oviposit in hosts, the motivation level will decrease steadily over time down to a threshold value, whereupon the parasitoid leaves the patch. However, with each oviposition that occurs, an incremental change in motivation occurs. The initial level of motivation, combined with linear decreases of motivation during searching periods and increases in motivation following ovipositions, determines how long the parasitoid will stay in the patch (Fig. 1.5a). The causal hypothesis assumes there is an additional effect of a rejection of a parasitised host, causing a decrease in motivation level (Fig. 1.5b). Like the functional hypothesis, the causal hypothesis predicts shorter patch residence times with increasing patch exploitation, all other things being equal.

A rigorous test of the causal hypothesis ought to demonstrate whether the mechanism by which shorter patch residence times come about is an increase in the tendency to leave the patch after a rejection of a parasitised host. Such a test implies that one is able to assess the relative effects on the motivation to search of ovipositions in unparasitised hosts (i.e., increments), of the time interval between encounters, and of rejections of parasitised hosts (i.e., decrements). To illustrate how difficult it is to determine whether the rejection of parasitised hosts causes a decrease in the motivation to search, we will discuss in some detail the experimental evidence given by van Lenteren (1991). In one experiment, individual females of *Leptopilina heterotoma* were allowed to search on a 1 cm diameter patch of yeast containing four unparasitised hosts and sixteen parasitised hosts. Each unparasitised host parasitised during the experiments was immediately replaced by an unparasitised one. As a control, single females of *L. heterotoma* searched a similar patch containing only four unparasitised hosts, and any unparasitised hosts parasitised during the experiment were replaced by unparasitised ones.

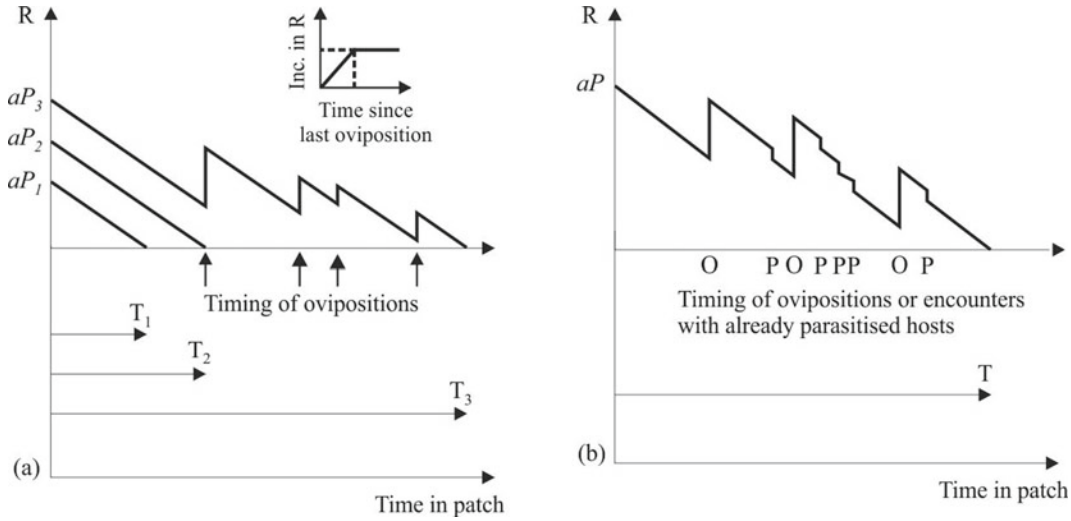


Fig. 1.5 Patch time allocation by parasitoids. **a** Waage's (1979) causal model of patch residence time. R = responsiveness of the female parasitoid to the patch edge (a function of the number of hosts in the patch [P_1, P_2, P_3] and a constant a, the quantity of kairomone produced per host). An oviposition results in an increment of R (inset: the size of the increment depends on linearly on the amount of time that has elapsed since the previous oviposition, and the increment cannot exceed a maximum value). T_1, T_2 and T_3 are the resulting patch residence times for three different cases. **b** Waage's (1979) model, modified to incorporate the decremental effect of encounters with parasitised hosts. Symbols as in (**a**) except that O denotes an oviposition, and P denotes an encounter with an already parasitised host. *Source* **a**: modified from Waage (1979), reproduced by permission of Blackwell Publishing; **b** modified from van Alphen (1993)

Wasps stay longer on patches with four unparasitised hosts than on patches with four unparasitised hosts and sixteen parasitised hosts. Van Lenteren (1991) argued that because there were no significant differences in average time interval between ovipositions in unparasitised hosts in the two treatments, the differences in patch residence times between the treatments can be attributed only to a detrimental effect on patch residence time of encounters with parasitised hosts. First, consider whether it is at all valid to conclude from the observation that average time intervals between ovipositions did not differ between experiment and control, and that there is no difference in the effect of ovipositions on patch residence time between the two treatments. This conclusion would be valid only if the parasitoid itself uses average intervals to assess patch profitability. As Haccou et al. (1991) have shown, the effect of an oviposition on the probability of a wasp leaving a patch depends on its timing; hence it is also important when and where the longest intervals occur. Despite a lack of statistical differences between the average values, important differences in interval times between ovipositions could occur between test and control treatments. An alternative explanation for van Lenteren's (1991) results is that the differences in patch residence time are caused solely by the decrease in motivation over time that results from the extra time spent in rejecting parasitised hosts in the treatment with parasitised hosts. This time could otherwise be spent in ovipositing in unparasitised hosts. Rejection of a parasitised host takes between 2 and 6 s (Haccou et al., 1991), and with on average 33 rejections in the control treatment, this behaviour may account for an important part of patch residence time. If the decrease in the motivation to search (indicated by the sloping lines in Fig. 1.5a, b) continues during the time spent in rejections, these small decrements may accumulate over time, causing the parasitoid to reach the threshold motivation rate for patch leaving sooner than when no parasitised hosts are encountered. Intervals between encounters with unparasitised hosts would, on average, be slightly longer in experiments with parasitised hosts than in those without them, as indeed they were: 84 compared with 79 s. Although these differences are not significant, the time lost in rejection of parasitised hosts gradually accumulates, and so may be responsible for the ultimate differences in patch residence times.

Clearly, one cannot test the causal hypothesis simply by determining whether patch residence times and search times differ significantly between treatments. What is required is an analysis in which the relative weight of effects of the influencing factors and their timing are estimated from the data and tested statistically. For this reason, several authors (e.g., Haccou et al., 1991, Wajnberg et al., 2003, 2004; Wajnberg, 2012, see Wajnberg, 2006 for a review) used Cox's (1972) proportional hazards model (Sect. 1.2.1) to analyse experimental data. Haccou et al. (1991) analysed a new set of experimental data using the model. No effect of encounters with parasitised hosts on the probability of patch leaving was found. If such an effect exists at all, we expect it to be a small one. It might be detected in experiments in which there is a high proportion of encounters with parasitised hosts (as this was the case, e.g., in Wajnberg et al., 2003, 2004, 2006). The first evidence confirming the hypothesis first formulated by Waage (1979) comes from Hemerik et al. (1993) who used the proportional hazards model to analyse their experimental results and demonstrated in female *Leptopilina clavipes* that encounters with parasitised hosts decrease the tendency to search the patch. Finally, the effect of encounters with parasitised hosts may depend on the previous experience of the parasitoid. It is thus possible that encounters with parasitised hosts could also increase the tendency to search on a patch, as is the case when they decide to superparasitise (van Alphen et al., 1987).

More recently, using a proportional hazards model to analyse patch residence time in *Trichogramma chilonis* females, Wajnberg (2012) discovered that each attack on a host had a significant incremental influence on the tendency of the females to leave patches of *Ephestia kuehniella* eggs. However, such effect depended on the sex of the egg females laid in each host attacked. Laying a daughter had a strong effect while laying a son had no effect. First of all, this indicates that

all previously published studies that were carried out with mated arrhenotokous females and that demonstrated an effect of each host attack should be re-analysed to see whether such effect was due to laying a son or a daughter or both. Moreover, *T. chilonis* females typically lay their sons first ('male-first' strategy; see Sect. 1.11.5). Using a modelling approach, Wajnberg (2012) demonstrated that the result obtained likely enables the females to adjust simultaneously their optimal patch time and sex allocation strategy according to LMC (Sect. 1.11.2).

Studies of patch residence times of insect predators are rare. It is evident that patch residence time may be influenced by the predator's level of satiation, but this is unlikely to be a straightforward relationship. For example, wolf spider (*Schizocosa ocreata*) patch residence time is influenced by hunger, but only in an interaction with spider age and sex (Persons, 1999).

1.5.4 Genetic Variation in Patch Time Allocation

As discussed above, several authors used Cox's regression model to investigate the patch-leaving behaviour of parasitoid wasps (see Wajnberg, 2006, for a review). Using this technique, Wajnberg et al. (1999) studied the behaviour of *Telenomus busseolae*, attacking the eggs of *Sesamia nonagrioides*. Not only did they find that female *T. busseolae* increased their tendency to leave a patch after each successful oviposition attempt, but, using the isofemale lines methods, they also demonstrated that the genotype of the ovipositing female influenced this behaviour. However, most workers consider patch-leaving (and indeed most parasitoid or predator behaviour) rules as a species-specific trait (Driessen et al., 1995; Wajnberg et al., 1999), rather than a variable characteristic among the individuals under study. This is rather short-sighted in many ways, as it assumes: (1) that all populations of a given species will respond in a similar way to different hosts or patches, and (2) that the trait is fixed, whereas it is likely that there is heritable variation for the trait, and that natural selection may change the response found in a population over time.

1.6 Host and Prey Location Behaviour

1.6.1 Introduction

With the exception of ambush predators, insect predators and parasitoids employ a heirarchy of behaviours that enable them to locate and choose their prey. These behaviours are generally associated with either:

1. Finding the host or prey habitat
2. Finding the host or prey itself.

Within each of these levels, which, of course, are part of a continuum and are only delineated for our convenience, individual parasitoids and predators will generally follow a behaviour pattern that responds to cues. While such a scheme may allow us to visualise the foraging process, it must be remembered that these behaviours will be influenced by learning (a plastic response to experience) and genetic variation (both within- and between-population variation in responses to cues).

During searching, two important types of cue will influence insect natural enemy behaviour. Attractant stimuli induce a change in forager behaviour that results in orientation to areas that either contain, or are likely to contain, hosts. Arrestant stimuli act by eliciting a reduction in the distance or area covered per unit time by the forager within such areas. These stimuli can act at a number of scales, with distinct cues influencing the behaviour of the forager over differing distances.

1.6.2 Host and Prey Habitat Location by Parasitoids and Predators

The literature concerning host habitat location derives largely from studies showing which stimuli (cues) attract parasitoids and predators to

the host's habitat (reviewed by Vinson, 1985). Few studies deal with functional aspects of this step in the foraging sequence (but see, e.g., Le Ru and Makosso, 2001; Gohole et al., 2003). The emphasis on causal aspects of host habitat finding reflects the fact that it is much easier to answer qualitative questions, such as which odour acts as an attractant, than it is to answer the question of why one odour should be attractive, and another not, in terms of the contribution to fitness of the insect natural enemy.

Parasitoids spend a significant proportion of their adult lives searching for places where hosts can potentially be found. They may use visual, acoustic or olfactory cues to locate potential host patches. Certainly, for parasitoids, olfactory cues are more important. Often, visual and acoustic cues can guide a parasitoid to its host over a short distance only, in contrast to olfactory cues that can act over much longer distances.

It is difficult to demonstrate the use of visual cues in host habitat location by insect predators and parasitoids, because the use of other, olfactory and acoustic, cues must be excluded. Van Alphen and Vet (1986) investigated the searching behaviour of *Diaparsis truncatus*, an ichneumonid parasitoid of larvae of the twelve-spotted asparagus beetle, *Crioceris asparagi*. Larvae of the beetle feed inside the green berries of the *Asparagus* plant. It was shown, by placing green-painted wooden beads on *Asparagus* plants, that *D. truncatus* females respond from a distance to the berries of *Asparagus*. The parasitoids landed more often on the slightly larger wooden beads than on the green *Asparagus* berries, which is consistent with the hypothesis that the parasitoids respond to visual cues. Such an approach may be adopted for parasitoids of other insects living in fruits. Visual responses of parasitoids of leaf-rollers and stem-borers could be investigated by presenting females with paper tubes of various colours, sizes and shapes. In this case, it is useful to record the light absorption/reflectance characteristics of the objects used rather than simply their apparent colour.

Coccinellids are perhaps the best-studied insect predators (Dixon, 2000), and it appears that visual cues can play a large part in longer-distance prey location. Hattingh and Samways (1995) found that the ladybird *Chilocorus nigritus* initially orientated towards a simulated tree line, and then showed a preference for simple ovate leaves over more complex leaf shapes. Hattingh and Samways (1995) studied 'biotope' location behaviour using a flight chamber that comprised a transparent, Perspex cylinder, which was closed at both ends. The chamber itself was situated in a room whose walls and ceiling were covered with white paper. On each of the walls facing the chamber ends were screens, upon each of which was painted a particular image: vertical *versus* horizontal stripes, flat horizon *versus* horizon with a tree line, shape of a tree *versus* vertical stripes, shapes like citrus leaves *versus* squares. Sixty coccinellids were released, per replicate, into the centre of the chamber and the numbers of beetles at either end recorded for up to one and a half hours. To eliminate any bias towards either end of the arena, the chamber was rotated 180° between replicates. Significantly more beetles were recorded at the end of the chamber facing the images of a horizon with a tree line than at the opposite end with a flat horizon, and also significantly more were recorded at the end facing the citrus leaf images than at the end facing the squares. Most of the plants on which *C. nigritus* occurs in nature have ovate leaves. Overall, Hattingh and Samways (1995) found evidence that beetles habituate to visual cues, as predators and parasitoids are known to do for olfactory cues.

Some parasitoids respond to acoustic stimuli produced by the host, and so execute host habitat location and host location in one step, and this appears to be much more common among dipteran parasitoids (Feener & Brown, 1997). Cade (1975), whilst broadcasting the song of the male cricket *Gryllus integer* from a loudspeaker to study the mating behaviour of the crickets in the field, discovered that a tachinid parasitoid (*Euphasiopteryx ochracea*) of the cricket was attracted by the song. Burk (1982) similarly demonstrated this for the tachinid *Ormia lineifrons*. Soper et al. (1976), using tape recordings, showed that the sarcophagid parasitoid

Colcondamyia auditrix finds male cicadas by this means (phonotaxis). Phonotaxis by the tachinids *Ormia depleta* and *O. ochracea* has been demonstrated using synthesised male calling songs (Fowler & Kochalka, 1985; Walker, 1993; Adamo et al., 1995). Both Fowler (1987) and Walker (1993) carried out experiments in which the synthesised calls of a range of several host cricket species were simultaneously broadcast in the field. Allen (1998) found that the parasitoid *Homotrixa alleni*, an ormiine fly, locates the bushcricket *Sciarasaga quadrata* by orientating towards calling males. Gravid female flies were most likely to search when calling was maximal, and by using trapped male *S. quadrata*, it was shown that there was a positive correlation between call duration and the number of flies attracted to the bush crickets (Allen, 1998).

Chemical communication, both between insects and between plants and insects, plays a very important role in determining the behaviour of parasitoids and predators. Any chemical conveying information in an interaction between two individuals is termed an infochemical (Dicke & Sabelis, 1988). Infochemicals are divided into pheromones, which act intraspecifically, and allelochemicals, which act interspecifically. Allelochemicals are themselves subdivided into synomones, kairomones and allomones. A synomone is an allelochemical that evokes in the receiver a response that is adaptively favourable to both the receiver and the emitter; a kairomone is an allelochemical that evokes in the receiver a response that is adaptively favourable only to the receiver, not the emitter; an allomone is an allelochemical that evokes in the receiver a response that is adaptively favourable only to the emitter (Dicke & Sabelis, 1988). The majority of parasitoids and many insect predators respond to volatile kairomones or synomones in the long-distance location of their hosts. These chemicals may originate from (1) the host itself, e.g., from frass, during moulting, during feeding, sex pheromones and aggregation pheromones, i.e., the chemicals involved are kairomones for the parasitoids; (2) the host's food plant, i.e., the chemicals involved are synomones for the parasitoids; or (3) some interaction between host and food plant, e.g., feeding damage, i.e., the chemicals involved are synomones for the parasitoids.

The attraction responses by parasitoids to odours from any source can be studied using various olfactometers, wind tunnels and locomotion compensators (servospheres), or by observing the responses of parasitoids to odour sources following release of the insects in the field.

Olfactometers

Two types of airflow olfactometer are commonly used to study responses to olfactory cues. One is the glass or clear Perspex Y-tube olfactometer (Fig. 1.6). The insect can be given a choice either between odour-laden air (test) and odour-free but equally moist air (control) or between air laden with one odour and air laden with another odour. Although Y-tube olfactometers have been criticised because odour plumes may mix where the two arms of the olfactometer meet due to turbulence, and that choice is no longer possible once the insect has passed the junction of the tube, impressive results have been obtained. Smoke can be passed through the apparatus to test for unwanted turbulence, but tobacco smoke must be avoided as it is absorbed by the tubing and it can affect the outcome of future experiments. By passing NH_4OH vapour over HCL, a fine smoke of NH_4Cl crystals can be created and the vapour channelled through the Y-tube. After testing, the crystals can easily be washed from the tubing. Turbulence, if detected, can often be reduced by adjusting the flow speed of the air.

With diurnally active insects, a diffuse light source is often required to illuminate the apparatus to encourage the insects to move towards the fork of the tube. This light should not cause the olfactometer to overheat, and so to avoid this a cold-light source (e.g., fibre optics) ought to be used.

To eliminate the effects of any asymmetry in the apparatus, the chambers need to be alternated for each 'run'. It is recommended that parasitoids be tested individually, rather than in batches, because either interference or facilitation may occur between insects and so bias the results. The apparatus should be washed, first with alcohol

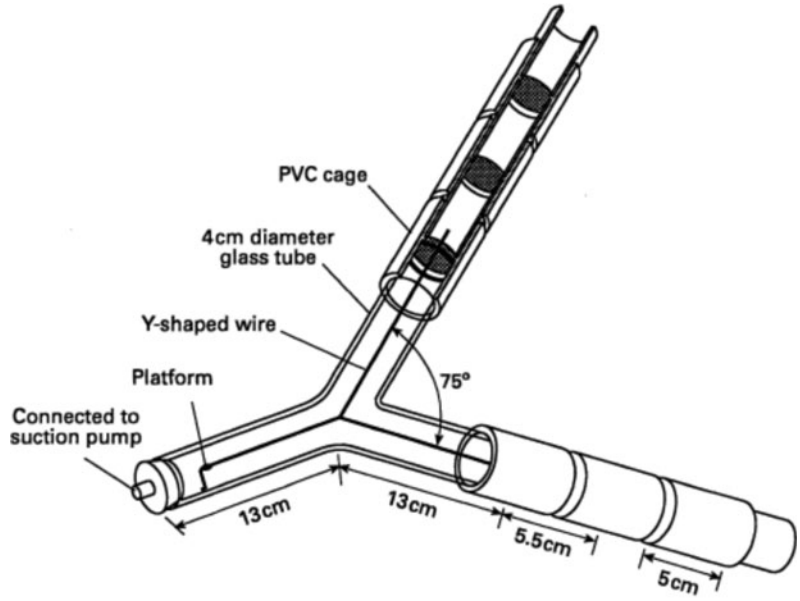


Fig. 1.6 Y-tube airflow olfactometers. The upper panel shows the design used by Sabelis and van de Baan (1983). The Y-shaped wire within the tube cavity provides a walking surface for small predators and parasitoids. For details of operation, see text. The lower panel shows a Y-tube olfactometer being used to assess parasitoid odour preferences. Odour emanated from two pieces of white filter paper, within the arms, which had previously been soaked in extracts of healthy or infested host-plant leaves. Note the regulators which ensured equal flow through the two arms (photograph K. S. Shameer)

and then with distilled water, between runs to prevent any response of parasitoids to any trail left by previous individuals. Finally, consideration needs to be given to the possibility of left- and right-handedness in the insects. By analysing the number of left and right turns in the apparatus, it is possible to test, statistically, whether wasps tend to move more to the right or more to the left. The null hypothesis will be that the distribution of turns by parasitoids should be equal in both arms irrespective of the position of the chambers. An additional test of turn preference is to perform several runs when both chambers are empty, although insects may be unwilling to move through the apparatus in the absence of any odour. Some parasitoid species

show 'handedness', i.e., a tendency to turn more in one direction than another (J. Pritchard, unpublished).

Even when great care is taken in the design of olfactometer experiments and the analysis of data, the results of olfactometry may be difficult to interpret (Kennedy, 1978). This applies especially to Y-tube olfactometers. The Y-tube, when employing a light source, simultaneously presents test insects with two types of stimulus, light and air current, to which the insect might respond by phototaxis and anemotaxis, but presents the two odours (or odour and non-odour) separately at only one point in the apparatus: the fork, which represents the 'decision point'. Responding by phototaxis and anemotaxis to the common air current, insects might be entrained past the decision point and become behaviourally trapped in the wrong arm (Vet et al., 1983).

One recent use of Y-tube olfactometery first tested the response of the parasitoid *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), an endoparasitoid of larvae of tomato pest *Tuta absoluta* (Lepidoptera: Gelechiidae), towards the headspace volatiles of healthy plants, pest-infested plants, pest larvae and larval frass, and then tested the individual odour attractants present in these (Ayelo et al., 2022). This showed that the pest larvae-infested tomato plants and the host larval frass volatiles were more attractive and that the parasitoids are specifically attracted to the terpenoids α-pinene, β-myrcene, α-phellandrene, α-terpinene, β-ocimene, (E)-β-caryophyllene, and to the benzenoid ester methyl salicylate.

Bertoldi et al. (2019) used two different types of Y-tube olfactometer setups to determine the behavioural responses of female *Trissolcus japonicus* to host-associated cues of *Halyomorpha halys* and *Podisus maculiventris*. In the 'long-distance' setup, the air streams passed through a 4-L glass jar (diameter: 10–15 cm; height: 30 cm) containing the odour source and connected to the olfactometer arm through a 40 cm-long plastic tube and, in the 'close-distance' setup, the sources of volatiles were placed close to the olfactometer in two small chambers connected with the tubes and placed directly at the ends of the olfactometer arms. Bertoldi et al. (2019) tested the volatiles from stink bug treated plants in the 'long-distance' setup, and those from adults and eggs of the hosts in both setups, because oviposition-induced plant volatiles may be perceived from a longer distance than the volatiles from adult stink bugs and from eggs (Conti et al., 2003; Colazza et al., 2010; Hilker & Fatouros, 2015).

Frati et al. (2008) used a vertical open Y-shaped olfactometer (originally developed to record aphid behavioural responses to plant odours by Visser & Piron, 1998; Fig. 1.7) to test the response of *Lygus rugulipennis* females to host-plant volatiles. This type of olfactometer is constructed from a brass rod positioned vertically and divided into two arms, over which two separate glass tubes are placed to direct either clean air flow or an air flow containing odour towards the Y-junction. The insect, initally placed on the base of the vertical rod, starts walking upward and chooses between the arms at the junction. For further studies using olfactometers, see, for instance Chiappini et al. (2012) and Rondoni et al. (2017a, 2017b).

Another type of airflow olfactometer, designed by Pettersson (1970) to study the responses of aphids, avoids many of the disadvantages of Y-tube olfactometers. A modification (Fig. 1.8) of the Pettersson olfactometer by Vet et al. (1983) and further developed by others (e.g., Sengonca & Kranz, 2001) has been widely used to analyse parasitoid behaviour. It is constructed mainly of transparent acrylic (Perspex, Plexiglas) and has a central arena with four arms. Air is drawn out of the arena via a hole in the centre of the bottom plate. Air flows into the arena via four arms. Insects in the central alrea may therefore be exposed to as many as four different odours. Air speed in each arm can be controlled with a valve and an anemometer and should be equal in all arms. Before an experiment is performed, an NH_4OH smoke test can be carried out to test for unwanted turbulence and to show that a clear, straight boundary exists between odour fields. Diffuse light of equal intensity on all four sides of the arena prevents asymmetric attraction of insects to light.

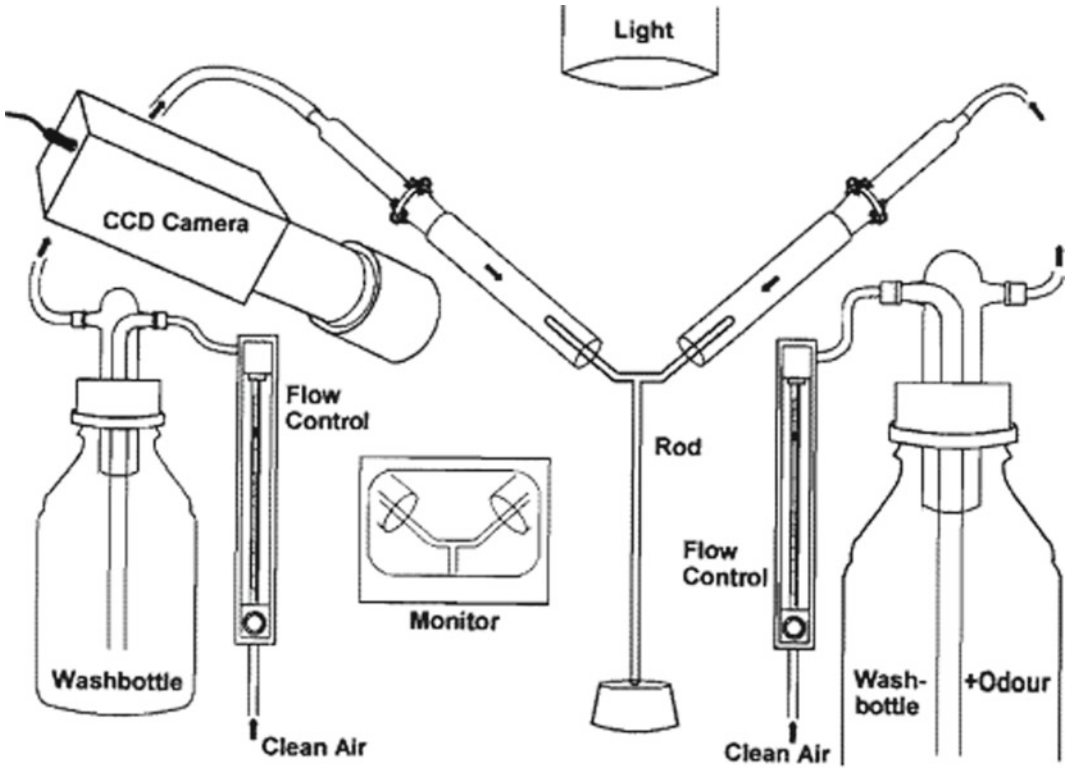


Fig. 1.7 Open Y-track olfactometer. The insect is placed on the base of the vertical rod and starts walking upward. At the junction it will choose between a clean air flow, here at the left, and an air flow loaded with plant odour, shown at the right. Subsequently the insect is removed and the experiment repeated with another insect (reproduced from Visser & Piron, 1998, with permission from the Netherlands Entomological Society)

Insects are introduced through a hole in the bottom plate by temporarily disconnecting the tube from the air pump. Observations are best made using a video camera placed directly overhead, because any movements by a human observer may disturb the insects. Such systems can be further automated by integrating commercially available components, such as CCD (charge-coupled device) cameras, with a computer program that incorporates a positioning and tracking algorithm (Vigneault et al., 1998; Sect. 4.2.6).

The Pettersson olfactometer thus allows an insect to choose between four different odour fields, and repeated choices by the insect are also made possible. In non-automated versions of the Pettersson olfactometer, the final choice by an insect is usually considered to be made when it enters the narrow tube through which air laden with odour enters the arena. Both because airflow in this narrow region is strong and because many parasitoids have an aversion to entering narrow crevices, some insect species avoid this area and turn without entering. Other parasitoids react to the odour stimulus by flying vertically upwards. Because flight is impossible in the narrow space between the base and the olfactometer cover, the insects will hit the top plate, and after a number of these aborted flight attempts become so disturbed that they cannot be expected to choose odour fields.

Often, one or more of the odours offered in an olfactometer comprises a mixture of many unidentifiable volatile substances, the concentrations of which in the odour fields are unknown. This does not pose a problem if the responses of an insect to a mixed odour source and a clean air control are compared, because

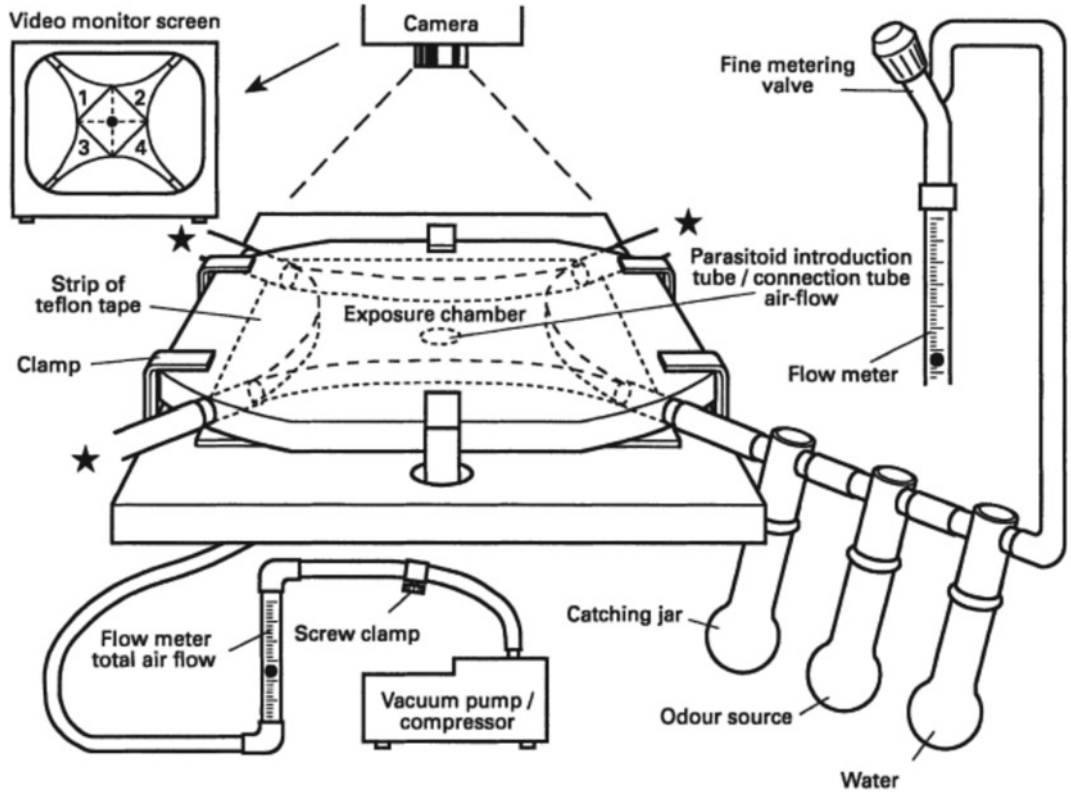


Fig. 1.8 Design of the Pettersson olfactometer, as used by Vet et al. (1983). The catching jar is used to collect any insects that move into an outflow tube. The stars represent three further odour sources that are not shown. Reproduced by permission of Blackwell Publishing. For a relared 6-arm design see Aleosfoor et al. (2014)

only the test odour is the potential attractant. However, when testing for attraction to two odour sources (e.g., the odours of two different food plants of the host), there may be problems of interpretation. One of the odour sources may be more attractive than the other because the insect responds to one or more substances in that odour source that are lacking in the other. Alternatively, both odour sources may be qualitatively similar but the insect may be differentially attracted because of differences in the concentration of an attractant component of an odour. It also needs to be borne in mind that a combination of a qualitative difference and a quantitative difference may be responsible for differential attraction. The ultimate solution to the above problem would be to isolate the attractants and test whether differential attraction to odour sources is due either to differences in chemical composition between the sources or differences in concentration of their chemical components.

With any airflow olfactometer it is important to ensure, before carrying out any experiments, that air flows through the apparatus at a constant rate (usually the rate is low). With the Y-tube and Pettersson olfactometers, both of which are hypobaric systems (i.e., air is sucked out), a good-quality vacuum pump should be used. Flow meters of the correct sensitivity, i.e., neither over- nor under-sensitive, should also be employed. Static-air olfactometers (i.e., without generated air flow) can be used with predators and parasitoids to measure chemotactic responses to odour gradients (Cusumano et al., 2022a, 2022b; Steidle & Schöller, 1997). One such olfactometer, used successfully by Vet (1983), consists of three chambers (Fig. 1.9): the

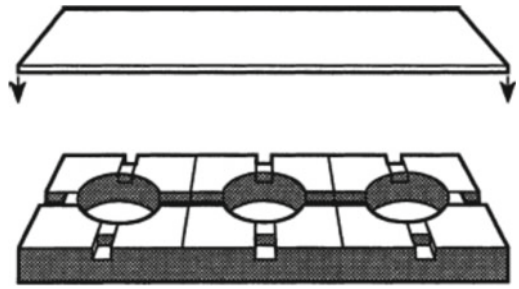


Fig. 1.9 The static-air olfactometer of Vet (1983). The apparatus comprises three Perspex blocks glued together and covered by a single glass lid (a single Perspex block would also suffice, although it may be more difficult to excavate). The excavations (chambers) in the blocks are connected by corridors. The chambers, measured internally, are 50 mm wide and 16 mm deep; the corridors are 10 mm wide and 5 mm deep. Reproduced by permission of E.J. Brill (Publishers) Ltd

parasitoid or predator is released into the middle chamber and its subsequent choice of outer chamber containing a test odour recorded. Vet (1983) also recorded the time taken for females to reach an odour source chamber.

Static olfactometers can also be used to evaluate whether the parasitoid is able to perceive the stimuli as short-range volatile cues (Conti et al., 2010). This is similar to the closed arena experiments, the only difference being a fine mesh placed between the leaf surface and the observation chamber, to prevent direct contact between the parasitoid and the leaf surface. Conti et al. (2010) found that, in the static olfactometer, *Trissolcus brochymenae* reacted to short-range volatiles from cabbage leaves with feeding damage, oviposition and walking paths by *Murgantia histrionica*. Parasitoid arrestment behaviour to the host-insect cues can be investigated in an open arena made of filter paper (185 mm diameter) which allows the parasitoids unconstrained movements (Bayram et al., 2010). The central circular area (10 mm diameter) in the open arena was contaminated with the abdominal scales of virgin females of *Sesamia nonagrioides* and the arrestment responses of naïve female wasps of *Telenomus busseolae* were recorded using a monochrome CCD video camera. A similar bioassay was used by Bertoldi et al. (2021), but on a large filter paper arena (20 × 20 cm), to assess the behaviour of *Telenomus podisi* to host-associated cues of female *Halyomorpha halys* and *Podisus maculiventris*.

Locomotion Compensators

The analysis of insect behaviour in response to semiochemicals can be difficult when using olfactometers, as the details of walking tracks may not be observable and may be influenced by the confines of the apparatus (Visser, 1996a, 1996b). Locomotion compensators (also called servospheres or trackspheres) for insect studies were first constructed and described by Kramer (1976) and are used for measuring the orientation behaviour of a variety of insects, such as aphids, moths, beetles, bugs, cockroaches, crickets, honeybees and parasitoid wasps (Thiery & Visser, 1986; Vet & Papaj, 1992; Visser, 1996a, 1996b; Geiselhardt et al., 2008; Rouyar et al., 2011; Minoli et al., 2012; Party et al., 2013; Piesik et al., 2013).

The instrument consists of a sphere, on top of which the insect is placed, and which is rotated opposite to the insect's displacements by means of two electric motors (Fig. 1.10). The motors are driven by electrical commands proportional to the displacement of the insect measured by an optical detector (Video CMOS camera with Macro zoom lens) located above the insect. As a result, the insect stays on top of the sphere while walking. The locomotion compensator permits unconstrained movement and thus avoids the behavioural artefacts commonly encountered in more confined experimental setups. The rotational movements of the sphere are detected by two encoders or pulse generators in contact with the sphere. The speed and direction are calculated every second and transmitted to a computer, where the displacements are stored as incremental X and Y coordinates. The computer software program collects and stores the displacement data, reconstructs the walking path and provides track analysis (Vet & Papaj, 1992). The following four track parameters can be used to quantify the insect's behaviour: (1) walking

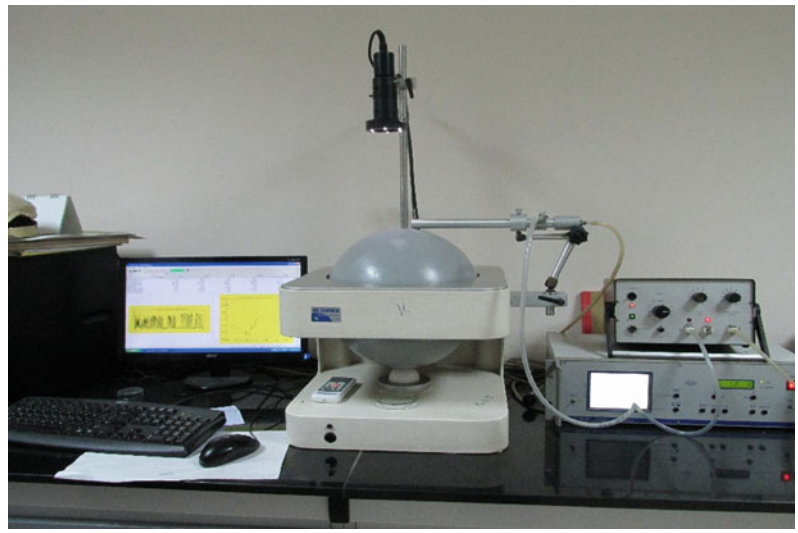


Fig. 1.10 A locomotion compensator being used to test the responses of host larvae to plant volatiles (photograph K. S. Shameer). Examples of parasitoid walking tracks can be found in Vet and Papaj (1992)

speed (mm/s); (2) straightness of walking, i.e., the ratio of vector length to total track length (ranging from 0 to 1); (3) upwind length (mm), i.e., the net distance from the origin towards the odour source along a straight line; and (4) upwind fixation, the ratio of upwind length to total track length (ranging from −1 to +1).

Vet and Papaj (1992) used a locomotion compensator to study the effect of oviposition experience on the upwind movement of the eucoilid parasitoid, *Leptopilina heterotoma* (Thomson), in odour plumes of host microhabitats. The parasitoids were first exposed for 2 h to the host *Drosophila melanogaster* larvae in either fermenting apple yeast or decaying mushroom substrate. They found that females experienced with a particular substrate walked faster and straighter, made narrower turns and spent more time in upwind movement toward the source in a plume of odour, whereas inexperienced females showed little or no difference in response to alternative odours.

Some workers use several bioassay methods together to study the orientation behaviour of insects to host-plant volatiles, pheromones, etc. For instance, Tinzaara et al. (2003) used three different bioassay methods, a locomotion compensator, a dual port olfactometer and double pitfall olfactometer, to study the orientation behaviour of *Cosmopolites sordidus* to host-plant volatiles and a synthetic pheromone. The results of all three bioassays indicate that *C. sordidus* responds in an additive way to the combination of plant volatiles and the synthetic pheromone.

Wind Tunnels

As noted above, not all parasitoids can be successfully tested in olfactometers, because they are prevented from flying. Flying parasitoids can be tested in wind tunnels (Drost et al., 1986; Keller, 1990; Parent et al., 2017; Fig. 1.11), but it is difficult to keep track of the smaller species. Wind tunnels allow the parasitoid wasps to express their full range of behaviour, especially the searching behaviour towards their host insects, and it also allows observation of the wasp's preflight behaviour, flight behaviour and behaviour after landing on the host (Yazdani et al., 2015). Wind tunnel trials showed that *Aphidius ervi*, a common parasitoid of the pea aphid, *Acyrthosiphon pisum*, preferred to fly towards aphid-damaged bean plants (Du et al., 1996; Powell et al., 1998; see also the discussion below). Wind tunnel experiments also found that flight duration and the profile of foraging behaviours exhibited by the parasitoid *Goniozus jacintae* depended on the instar of the host light brown apple moth, *Epiphyas postvittana*, a lepidopteran pest of grapevines (Aspin et al., 2021). Using the same wind tunnel, Yazdani et al.

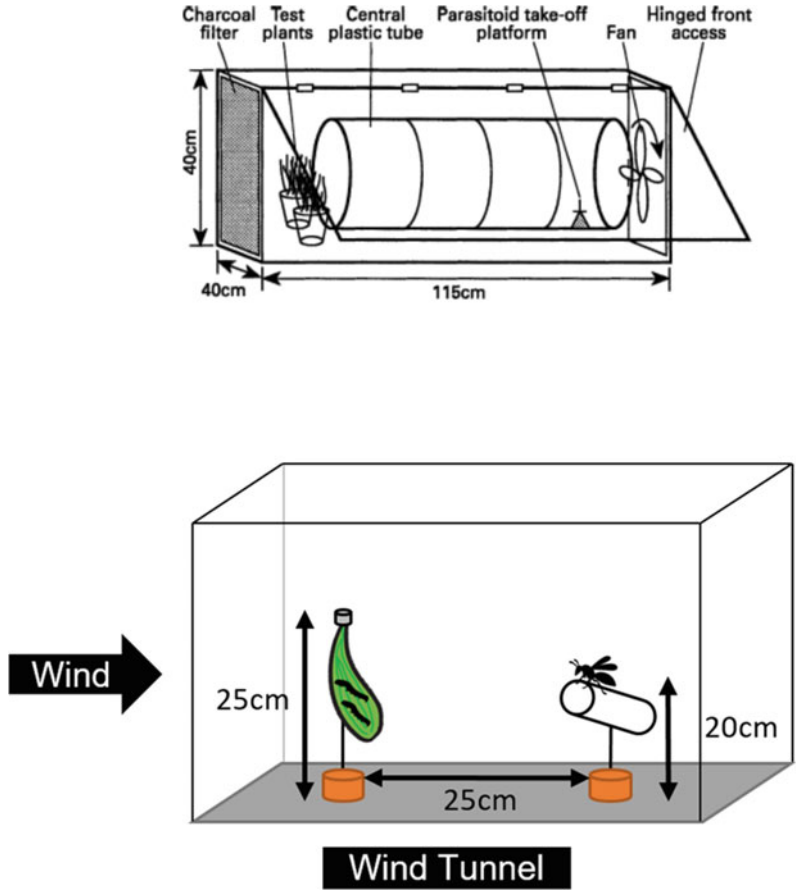


Fig. 1.11 Wind tunnels. Upper panel: Design of the wind tunnel used by Grasswitz and Paine (1993) to study the behaviour of *Lysiphlebus testaceipes* (Hymenoptera: Braconidae), a parasitoid of aphids. The main (rectangular) chamber was constructed of Plexiglass, and the central (cylindrical) test section was constructed of Mylar (biaxially-oriented polyethylene terephthalate). Lower panel: Schematic diagram of the wind tunnel used by Aspin et al. (2021) to study the sequence of forgaging behaviours of female *Goniozus jacintae* (Hymenoptera: bethylidae), a parasitoid of lepidopterans attacking grape vines and plantains. Host larvae fed on plant leaves that were suspened from a bar fixed 25 cm above the floor of a wind tunnel and wind speed was set at 20 cm s^{-1}. In each trial, a single female wasp was released 25 cm downwind from the infested leaf

(2015) found similar results for a different parasitoid, *Dolichogenidea tasmanica*, of the same pest species.

Other Methods and Further Considerations

When a wind tunnel cannot be used, one could try the following:

1. Placing potentially attractive odour sources in an array, either in the field, in a large field cage, or in a large controlled environment chamber.
2. Releasing a large number of adult females and examining the odour sources frequently.
3. Removing each insect that lands on the odour sources.

If more individuals than expected, based on a random distribution, land on a particular odour source, this can be taken as evidence that the odour source is attractive. If different odour sources are offered, it may be possible to rank them in terms of their attractiveness. The

problem with this type of experiment is that the number of parasitoids or predators trapped on a particular source is a function both of the number of insects landing on the source and of the time they spend there. Ideally, the test individuals should be caught immediately after arrival on the source, but this is not always possible. Another problem is that the experimental design does not exclude the effect of interactions between individuals. For instance, parasitoids may repel conspecifics, even actively chasing them away (Hardy et al., 2013).

The field release method (involving counting of the numbers of females attracted to uninfested and infested cassava plants) was used successfully in field experiments with *Apoanagyrus* (= *Epidinocarsis*) *lopezi* and to compare the attractiveness of different microhabitats containing *Drosophila* larvae to several species of *Leptopilina*. The method also allows a functional analysis of habitat choice. If one knows (1) the encounter rates with hosts in the different microhabitats, (2) the species composition of the host larvae in each microhabitat, and (3) the survival rates of parasitoid eggs deposited in each of the host species, one can calculate the relative profitability of each microhabitat for the parasitoid and then predict which ones the parasitoids should visit when given a choice. This approach was employed by Janssen et al. (1991).

Another approach to studying functional aspects of host habitat location by parasitoids is to consider the reliability and detectability of a cue (Vet & Dicke, 1992). This approach contrasts cues having a high detectability but a low predictive value regarding the presence of hosts, with cues having a low detectability but a high predictive value. Cues with a high detectability are odours emitting from potential host plants. Cues with a high reliability are, for example, substances produced by the host plant in reaction to the presence of the host and substances emitted directly from the host. Vet and Dicke (1992) assumed that high reliability cues are produced in smaller amounts than general host-plant odours. Measuring reliability and detectability in a quantitative way is a problem in testing the concept. However, a number of studies (discussed below) have gone some way to overcoming this problem. A related concern is highlighted by McCormick et al. (2014a), who caution that there could be minor volatile cues, seen as little peaks in chromatography (indicative of low volumes), released from host plants but with important roles in plant–insect interactions, and describe experimental approaches and chemical and statistical methods to detect these minor compounds with major biological activities.

As with all behavioural tests that purport to investigate insect preferences (e.g., for a given odour, host plant, host size, host instar, etc.), statistical concerns abound. Two will be considered here. First, at which point is the test insect provided with too many choices to make effective comparisons and how does this affect the sample size required? In part, the answer depends on whether the experimenter is interested in extremes (whether an insect prefers an odour to the control treatment) or in forming a rank order of preference among odours. Raffa et al. (2002) show that an increased number of replicates is required to show the latter and they provide an excellent guide as to how to maximise experimental power (see also Taborsky, 2010; and Smith et al., 2011, for general discussions of sample sizes and power). Second, what is the best way to analyse the data? There are several established statistical approaches. Data from binary outcomes can usually be analysed using a standard probit or logistic regression approach (Chap. 9) but standard probit models are not suitable for preference assays, and Sakuma (1998) provides an extension of the standard probit method to overcome these problems.

Many studies have shown that parasitoids and insect predators respond to odours produced by the host plants of their potential prey or hosts (reviewed by Vet, 1999). In some cases, parasitoids respond to the odour of host-free (undamaged) plants, but frequently herbivore damage is required before a response to plant cues is observed. Wind tunnel trials showed that *Aphidius ervi*, a common parasitoid of the pea aphid, *Acyrthosiphon pisum*, preferred to fly towards aphid-damaged bean plants. However,

washing the plants to remove aphid cues did not reduce this preference, indicating that induced plant volatiles were being used as cues (Du et al., 1996). When extracts of the plant volatiles were applied to filter paper and placed in a wind tunnel, a similar effect was seen (Powell et al., 1998). If *A. ervi* females are provided with a choice between volatiles collected from pea aphid-damaged plants or from black bean aphid (*Aphis fabae*)-damaged plants, they are much more likely to fly towards the former (Powell et al., 1998). This is evidence that there are host-specific cues in the plant volatiles (Du et al., 1996; Powell et al., 1998; Costa et al., 2010). *Dolichogenidae tasmanica* responded to the volatile cues associated with two related tortricid host species, *Epiphyas postvittana* and *Merophyas divulsana,* in a dual-choice wind tunnel experiment (Bui et al., 2020).

Predators also respond to similar cues. In a field experiment, Drukker et al. (1995) found that psyllid-infested pear trees attracted significantly more anthocorid predators than uninfested trees. Scutareanu et al. (1997) collected volatiles from the headspace (i.e., the air directly above the leaf) of attacked and unattacked trees, and using a Y-tube olfactometer found that the anthocorids preferentially chose the airstream containing volatiles from attacked trees. Using mass spectrometry, they found six volatiles that were significantly more common in the headspace of attacked trees (the monoterpene (E, E)-α-farnesene, the phenolic methyl salicylate, and four green leaf compounds). Only the monoterpene and the phenolic compounds elicited the preference in the bugs (Scutareanu et al., 1997). Methyl salicylate has also been shown to influence the behaviour of other predatory arthropods, including phytoseiid mites (Dicke et al., 1990), and anthocorids are attracted to methyl salicylate produced from mite-infested beans (Dwumfour, 1992). However, not all increases in volatile emission following herbivore attack led to an increase in natural enemy recruitment. For example, induced secondary defences in cucumber plants correlate with an increase in volatiles, and this deters predatory mites (Agrawal et al., 2002).

It has been suggested that plants might produce synomones (herbivore-induced plant volatiles, HIPV) as an indirect defence. By actively recruiting natural enemies of their herbivores, the damage suffered by the plant will be reduced over time (Vinson, 1999). This hypothesis is not without critics: van der Meijden and Klinkhamer (2000) point out that plants may not benefit from the presence of koinobiont parasitoids (Sect. 1.6.7), since they do not immediately kill the host and damage continues to occur after the host is attacked. More direct benefits accrue to the plant from the recruitment of predators, and parasitoids may simply be subverting this interaction. The quantity and composition of the volaitles produced by plants may vary with the herbivore species, the plant species and the environmental conditions under which plants are grown. In principle such emissions may be an adaptive response by plants to minimise damage by herbivores (Hare, 2011). The argument that plants benefit most from natural enemies that quickly kill herbivores or cause them to immediately cease feeding (Faeth, 1994; van der Meijden & Klinkhamer, 2000) is substantiated by Hare (2011): predators, which generally have broader diet ranges than parasitoids, and idiobiont parasitoids that immediately terminate herbivore feeding have greater impact on plant fitness than koinobiont parasitoids.

Synomone production by a herbivore-infested plant will depend on the feeding mechanism of the herbivore species. Maize plants attacked by the aphid *Rhopalosiphum maidis* (a phloem feeder) do not increase volatile production, whereas the lepidopteran *Spodoptera littoralis* (a leaf-chewer) and the stem-borer *Ostrinia nubilalis* induced large changes in volatile production (Turlings et al., 1998). However, plants attacked by *O. nubilalis* released a *lower* quantity of volatiles, and these included several unidentified volatiles that were not induced by *S. littoralis* attack, supporting the hypothesis that herbivore-specific volatiles may be produced by infested plants. The differences in HIPV induction by different herbivores can be very precise but are sufficiently distinct to be recognised by the natural enemies (Turlings & Erb, 2018), and this

allows specialist parasitoids to locate plants that are attacked by their specific hosts (de Moraes et al., 1998; Mumm & Hilker, 2005; Schroder & Hilker, 2008; Webster et al., 2010) and suitable host stages (Takabayashi et al., 1995). Cultivated tobacco, cotton and maize produced qualitatively and quantitatively different HIPV blends when attacked by *Heliothis virescens* and *Helicoverpa zea,* and the specialist parasitoid *Cardiochiles nigriceps* preferred plants damaged by its host, *H. virescens*, to plants damaged by the non-host, *H. zea* (de Moraes et al., 1998). The HIPVs may also differ in response to feeding by different life-history stages of a single herbivore species, as naïve adults of *Cotesia kariyai* differentiated between the qualitatively and quantitatively different HIPV blends of maize induced by different instars of the *Pseudaletia* caterpillars (Takabayashi et al., 1995). Similarly, early and late instar *Lymantria dispar* caterpillars induce different patterns of HIPV emission from poplar trees, which may help the parasitoids to locate a suitable developmental stage of their prey (McCormick et al., 2014b). The infested leaves of coconut palms and frass of larvae produced many herbivore-induced plant volatiles that mediate both direct and indirect defences like attracting foraging carnivorous predators and parasitoids (Shameer et al., 2017).

Of course, a given herbivore species may not necessarily induce the same production of volatiles on different host plants, and different natural enemies may in turn show different responses to these plant-produced cues. A good example of this is provided by the work of Takabayashi et al. (1998) who studied two different tritrophic systems. In the first system, the parasitoid *Cotesia kariyai* was preferentially attracted to plant (corn) volatiles produced by damage from *Pseudaletia separata*. Surprisingly, this attraction was only elicited when the plant was attacked by early larval instars of the host; feeding by late instars did not induce any preference. Plants attacked by younger *P. separata* instars produce higher proportions of terpenoids and indole volatiles. As early instar *P. separata* parasitised by *C. kariyai* consume less leaf material than older larvae, it is beneficial for the plant if the herbivores are attacked when young (Takabayashi et al., 1995). But host age does not always influence parasitoid behaviour. Gouinguene et al. (2003) showed that *Microplitis rufiventris*, a parasitoid that cannot successfully attack first instar *Spodoptera litoralis*, could not distinguish among maize plants attacked by different instars of the herbivore.

In the second system, the congeneric parasitoid *C. glomerata* preferred volatiles produced by *Rorippa indica* (Cruciferae) plants infested with its host *Pieris rapae* over clean air. However, the parasitoid's preference is for artificially damaged plants over herbivore-damaged plants, although in both cases the plant releases (Z)-3-hexanol and (E)-2-hexenal. The technique used to artificially damage the plant seems to produce larger amounts of those volatiles. Takabayashi et al. (1998) suggest that the parasitoids in these systems use different mechanisms to overcome the reliability-detectability problem. *Cotesia kariyae* responded to volatiles that provide direct evidence of the presence of potential suitable hosts, whereas *C. glomerata* responded to volatiles produced in response to plant damage. *Rorippa indica* has few herbivore species, of which *Pieris brassicae* is one of the most prevalent, so responding to general damage cues is likely to lead the parasitoid to potential hosts.

There may also be differences in the response elicited by such cues between generalist and specialist predators or parasitoids. Röse et al. (1998) found that the specialist parasitoid *Microplitis croceipes* is attracted to insect-damaged cotton plants, whereas artificial damage (i.e., without herbivore kairomones) is enough to attract the generalist *Cotesia marginiventris*. In contrast, Dickens (1999) found that both generalist (*Podisus maculiventris*) and specialist (*Perillus bioculatus*) predators of Colorado potato beetle showed similar responses to the systemic volatiles produced by infested plants. The ability to recognise HIPVs that are associated with their specific hosts may be innate in specialist parasitoids (de Moraes et al., 1998), whereas generalist parasitoids learn to distinguish between different blends of volatiles so that they can focus on the most profitable cues (Vet et al., 1995). The damaged *Brassica*

plants release, in addition to other compounds, typical glucosinolate derivatives (Blaakmeer et al., 1994; Danner et al., 2015) which specifically attract parasitoids of herbivores that feed on the glucosinolate-producing plants (Neveu et al., 2002; Pope et al., 2008; Mumm & Dicke, 2010).

It is advisable to include at least three treatments when studying the potential role of insect herbivore host plants in natural enemy host location. These treatments are based on the following questions: First, does the predator or parasitoid respond to unattacked plants? Second, is artificial damage enough to generate a response (clipping using a sterilised pair of scissors or a hole-punch)? Third, does herbivore damage induce a response (allowing the herbivores to feed, before removing them and washing away any direct cues which may emanate from the host, such as frass)? In addition, one may ask if there is a synergy between host-plant and host-insect cues. Havill and Raffa (2000) showed that gypsy moth (*Lymantria dispar*) larvae fed on an artificial diet were not attractive to a foraging braconid parasitoid, *Glyptapanteles flavicoxis*, whereas caterpillars that had fed on their main host plant, poplar, were attractive.

As an aside, Rutledge and Wiedenmann (2003) attempted to alter the preference for different host plants in the parasitoid *Cotesia sesamiae*, a braconid parasitoid of stem-borers. *Cotesia sesamiae* preferentially attacks hosts in sorghum, and after four generations of artificial selection (attempting to obtain parasitoids with a preference for cabbage plants), no change was found in foraging behaviour. This suggests that there is little genetic variation (in this species at least) in response to plant cues in parasitoids.

Many species of insect herbivore communicate with conspecifics using infochemicals such as sex pheromones. These provide reliable cues to the presence of potential prey/host individuals. Pickett et al. (1992) identified and synthesised several aphid sex pheromones, and these have proved to be highly attractive to *Praon* spp. parasitoids in field trials (Hardie et al., 1994). However, it appears that other species of aphid parasitoid (*Aphidius ervi* and *A. eadyi*) do not respond to these cues in field-sited pheromone traps. This may result from the behaviour of the parasitoids; these species do not appear to fly towards point sources (Stowe et al., 1995). This may explain why aphids placed on plants near sex pheromone sources in the field suffered significantly greater parasitism than those aphids kept away from the odour source (Powell et al., 1998).

Using a combination of four-way olfactometer (Fig. 1.8) and Observer software (Sect. 4.2.6), Couty et al. (1999) found that *Leptopilina boulardi*, a parasitoid of *Drosophila melanogaster*, was attracted by a combination of the odours of both rotting fruit and a kairomone left by adult *D. melanogaster* on the substrate tested. Predators also respond to the presence of prey and plant odours. The black bean aphid, *Aphis fabae*, produces a kairomone that attracts *Metasyrphus corollae*, a predatory hoverfly (Shonouda et al., 1998), and the coccinellid *Hippodamia convergens* is attracted by plant cues released when the aphid, *Myzus persicae*, feeds on the plant (Hamilton et al., 1999). However, predators differ from parasitoids in that the host location behaviour may differ between the adult and larval stages, and also within the larval instars. Bargen et al. (1998) found that first instar larvae of the hoverfly, *Episyrphus balteatus*, were attracted to aphid cues, but not to honeydew. Older larvae did not respond to these volatiles, but aphid extracts, honeydew and sucrose did provide cues.

The olfactory responses of foraging parasitoids and predators may vary with age, nutritional state and experience. It is important to take account of these factors when designing experiments. Ideally, preliminary experiments should be carried out to test for any effects. Synovigenic species (Sect. 2.3.4) may spend the first few days of adult life searching, not for hosts, but for foods such as nectar and honeydew, which supply nutrients for egg development (Chap. 8). Therefore, when young or starved, they may be unresponsive to host plant and host odours. Some parasitoids may even be repelled very early in adult life by an odour, which later on in life is used in host finding. *Exeristes ruficollis* responds

in this manner to the odour of pine oil (Thorpe & Caudle, 1938). Such responses need not be fixed. In an elegant study, Lewis and Takasu (1990) showed that female *Microplitis croceipes* can learn to recognise different artificial odours associated with food and host sources. Starved individuals showed a preference for the odour associated with the food source, whereas well-fed females preferentially moved towards the host-associated odour.

Furthermore, the ecological context of the interaction may need to be considered. Orr et al. (2003) found that the likelihood of a phorid fly parasitoid successfully locating its ant host, *Linepithema humile*, depended on (host) interspecific competitive interactions. Successful host location was more likely when the host was interacting with a species that elicited a chemical, rather than a physical response. Le Ru and Makosso (2001) found that foraging coccinellid predators (*Exochomus flaviventris*) can distinguish between the odours of cassava infested with parasitised and unparasitised mealybugs, preferentially orientating towards the cassava-unparasitised mealybug complex. In a similar study, van Baaren and Nénon (1996) studied two mealybug parasitoids. Both are monophagous species, with *Apoanagyrus lopezi* attacking the cassava mealybug (*Phenacoccus manihoti*) and *Leptomastix dactylopii* attacking the citrus mealybug (*Planococcus citri*). Both parasitoid species readily responded to the odours of infested plants or unattacked hosts, but not to those produced by parasitised hosts. However, rather than the parasitoids ignoring the odour of parasitised hosts, it may be that parasitised hosts have an additional odour, which acts as a deterrent. Such work strongly suggests that simplistic approaches to tritrophic systems may underplay the importance of other species in altering the pattern of the interaction.

There is one frequently overlooked aspect of experimental design associated with studies of natural enemy responses to odours. Not only do the enemies themselves show both phenotypic and genotypic variation in response to cues, the plants and prey insects themselves show variation in the signal. For example, the parasitoid *Diaeretiella rapae* shows different responses to the volatiles released by two near-isogenic strains of *Brassica oleracea*, which differ only in the production of isothiocyanates (Bradburne & Mithen, 2000). Such results also hold across cultivars of the same plant species (Gowling & van Emden, 1994). Plants emitting terpenoids, a highly diverse group of compounds, show great variability in their emission among different genotypes (Gershenzon & Dudareva, 2007; Degenhardt et al., 2009). Each plant species and plant genotype releases its own particular blend of terpenoids (Degen et al., 2004) in different quantities and ratios in response to herbivore feeding and even the time of the day that feeding occurs (Loughrin et al., 1994; de Moraes et al., 2001; Shiojiri et al., 2006; Shimoda et al., 2012).

Genetic variation in kairomone production is also found among aphids. Not only will parasitoids show differential responses to different clones of aphids, the clones themselves will also show variation in response to alarm pheromone (Müller, 1983). It cannot be overemphasised that researchers studying aphid–natural enemy interactions should work with several different clones of aphids. To be pedantic, since aphids within a clone are for all practical purposes genetically identical, the clone is the replicate. Many studies of aphid–natural enemy interactions are essentially performed without replication, since only one clone is used.

There are many studies that show learning in parasitoid wasps. Cues that elicit no response in naïve females can induce a response when they have been experienced in association with host contact (e.g., Fukushima et al., 2002; Meiners et al., 2003). This is known as associative learning, defined as a response to a stimulus that usually does not induce a response, after that stimulus has been experienced in combination with another stimulus to which the animal already shows an innate response. The behavioural plasticity allowed by associative learning provides considerable flexibility in parasitoid foraging strategies.

Associative learning modifies the foraging behaviour in many parasitic wasps, as they adapt their responses to specific cues in accordance

with the rewards they receive (Costa et al., 2010). Female *Cotesia marginiventris* showed increased attraction to a specific plant odour in a six-arm olfactometer after the wasps perceived one of the herbivore-induced odours of three plants either (1) without contacting any caterpillars, (2) while contacting the host caterpillar *Spodoptera littoralis*, or (3) while contacting the non-host caterpillar *Pieris rapae* (Costa et al., 2010).

If *Aphidius ervi* females are allowed to experience oviposition on the plant-host complex (here the pea aphid, *Acyrthosiphon pisum*, on broad bean, *Vicia faba*), then they are significantly more likely to orientate towards the plant-host complex than naïve females. Naïve females will orientate towards a source of host volatiles (Du et al., 1996; the innate response), but experienced females will also show an increased response to volatile cues from uninfested plants, which is likely to be an example of associative learning (Guerrieri et al., 1997).

The effect of learning on behaviour may depend on the experience and physiological state of the parasitoid. Female *Leptopilina boulardi* (a eucoilid parasitoid of *Drosophila melanogaster*) will associate odour cues with host presence, increasing ovipositor searching when exposed to the cue (Pérez-Maluf & Kaiser, 1998). This increase in searching behaviour was not associated with mating or prior oviposition experience, although both factors did influence some parameters of host searching. Females with prior oviposition experience showed a higher latency and reduced probing duration, whereas mated females tended to have a reduced latency and increased probing duration (Pérez-Maluf & Kaiser, 1998). Female *L. boulardi* show heritable variation in these responses to learned cues (Pérez-Maluf et al., 1998).

The likelihood of learning appears to be related to the nature of the substrate the parasitoid is searching. Duan and Messing (1999) suggested that parasitoid acceptance of less preferred hosts may be more likely to change with experience, than if the parasitoid is allowed to learn cues associated with preferred host-substrate complexes. If this is the case, then it is possible that associative learning will not be equally likely to be found with all potential host species. Therefore, the absence of learning in one situation may not reflect what will be found with other potential hosts. For example, Morris and Fellowes (2002) found that natal host influenced the likelihood of learning in the pupal parasitoid *Pachycrepoideus vindemmiae*. Females that emerged from *Musca domestica* only showed a preference for that host species after gaining experience attacking it. Experience gained in attacking *Drosophila melanogaster* did not change their preference. In a similar manner, wasps that emerged from *D. melanogaster* only showed a preference for that host when allowed to gain experience in attacking *D. melanogaster* pupae and experience gained on *M. domestica* did not alter their preference.

In experiments investigating learning in parasitoids, it is often best to use novel cues, which can be controlled and measured by the investigator. In studies of associative learning, odours such as vanilla and strawberry essence have been successfully used. Iizuka and Takasu (1998) used this approach to show associative learning by the pupal parasitoid *Pimpla luctuosa*. In addition, they found that females ceased attacking dummy hosts which had the previously learned odour after several failed oviposition attempts, which suggests that parasitoids can also learn to ignore cues (Iizuka & Takasu, 1998). Similarly, female *Microplitis croceipes* that had antennated host frass or oviposited in a host in the presence of vanilla odour responded to the odour in the wind tunnel and the parasitoids oviposited in the presence of the odour responded to the odour even at 24 h after experience (Takasu & Lewis, 2003). This shows that oviposition in the host in the presence of odours strongly affects associative learning and the persistence of learned response to the odours (Takasu & Lewis, 2003; Giunti et al., 2015). *Lariophagus distinguendus*, the ectoparasitoid of *Sitophilus granarius*, after being trained by being kept on infested grains in the presence of an odorant furfurylheptanoate (FFH) preferred the odour field containing FFH in olfactometers (Müller et al., 2006). In this case, the reaction to FFH is caused by associative learning due to host experience as an

unconditioned stimulus, and this learning experience in wasps induced a memory equivalent to the long-term memory found in *Apis mellifera* and *Drosophila melanogaster* (Müller et al., 2006). Studies on the effect of associative learning of plant chemicals on host-searching behaviour in *Ascogaster reticulata*, an egg-larval parasitoid of *Adoxophyes honmai*, revealed that the female wasps conditioned with tea leaf preferred tea leaf over the other plant species (Seino & Kainoh, 2008). Similarly, Kawamata et al. (2018) studied the innate colour preference and associative colour learning ability of *Ascogaster reticulata*, a braconid parasitoid of the tortricid *Adoxophyes honmai*, and reported that the wasps trained to associate black or blue colour with the presence of a host egg mass showed increased preference for these colours.

1.6.3 Host Location by Parasitoids

Inferring Behaviour from Morphology

Perhaps one of the more straightforward means of deducing how a predator or parasitoid may locate its prey is to pay close attention to the insect's morphology. For example, pipunculid flies have extremely well-developed compound eyes, and in the females the forward-facing facets are considerably enlarged (Jervis, 1992), so it can be inferred that host finding in these parasitoids relies on vision (confirmed by Forbes P. Benton, see Waloff & Jervis, 1987). However, some caution should be used when inferring behaviour from morphology, and, ideally, the insect would be studied carefully to confirm that the trait does aid predation or parasitism. Nevertheless, a small amount of basic biology and natural history will provide a huge amount of help in understanding the species of interest.

Genetic Variation in Foraging Behaviour

Wajnberg and Colazza (1998) used a combination of automated recording and statistical techniques to study the foraging behaviour of the parasitoid *Trichogramma brassicae* and found not only that the searching efficiency of females within a patch determined the number of hosts they encountered, but also that there was significant genetic variation among females in this trait. Whether such genetic variation plays a role in allowing populations to adapt to different habitats is a question that deserves a great deal of attention (see Wajnberg, 2004, for a review). Van Nouhuys and Via (1999) studied variation among populations of the parasitoid *Cotesia glomerata* attacking small cabbage white butterfly (*Pieris brassicae*) caterpillars in wild and agricultural habitats. These habitats present very different environments to the foraging parasitoids, as in the agricultural habitat every plant that a parasitoid lands on may carry its host. Wasps that originated from the wild habitat tended to move more between plants, perhaps reflecting the spatial characteristics of wild host populations. Jia et al. (2002) found genetic variation in response to herbivore-induced plant volatiles in the predatory mite, *Phytoseiulus persimilis*. Isofemale lines (Sect. 3.2.3) showed a negative correlation between the likelihood of patch location and patch residence time, suggesting a trade-off between prey location and reproduction.

Kairomones

Having arrived in a potential host habitat, a parasitoid begins the next phase in the search for hosts. Often, insects show arrestment in response to contact with kairomones of low volatility deposited by their hosts on the substratum. Materials containing such kairomones (sometimes referred to as 'contact chemicals') have been shown to include host salivary gland or mandibular gland secretions, host frass, and homopteran honeydew and cuticular secretions. Several herbivore species have evolved traits which reduce the build-up of frass near to where they feed, reducing the likelihood of their location by foraging parasitoids. This is a relatively common behaviour in caterpillars dwelling in leaf shelters, who can eject their frass with considerable force, depositing it some distance from the potential host (Weiss, 2003).

Kairomones present on the host itself have also been shown to induce oviposition behaviour by several parasitoid species. For example, the

parasitoid *Aphidius ervi* shows strong responses to pea aphid (*Acyrthosiphon pisum*) siphuncle secretions, but only at very short range or on actual contact, and the presence of these kairomones alone is enough to induce oviposition behaviour (Battaglia et al., 2000). A similar response is shown by the parasitoid *Diadromus pulchellus*. This wasp responds to the presence of soluble polypeptides present in the cocoons of its host, the leek moth *Acrolepiopsis assectella* (Benedet et al., 1999).

Because stronger responses may be found to a kairomone after prior oviposition experience in the presence of the substance, an initial experiment ought to be performed using parasitoids with previous oviposition experience. The next series of experiments would involve comparing the response of parasitoids to patches of potential host habitat, within which hosts have never occurred (e.g., clean host plant leaves), with the response to patches within which hosts have previously occurred for some time. The following changes in behaviour might be observed in the searching insects: a decrease in walking speed, an increase in the rate of turning, a sharper angle of turn at the patch edge, an increase in the number and frequency of ovipositor stabs, an alteration in position of the antennae, an increase in the amount of drumming with the antennae and an increase in the amount of time spent standing still. Video-recording equipment, together with the computer software discussed in Chap. 4 (Sect. 4.2.6) can be used to record and analyse alterations in these behavioural components. Path tortuosity can be evaluated by measuring the angle between tangents drawn at intervals along the path.

A useful additional analysis that can be carried out involves designating areas of an arena, e.g., the kairomone-treated area and the clean area, and measuring the proportion of the total time available that the insect spends searching each area. If the parasitoid or predator can be shown to have spent a greater proportion of its time in the treated area, then it can be considered as having been arrested by the kairomone.

Once it has been demonstrated that patches within which hosts have occurred contain a stimulus to which parasitoids respond by arrestment, further experiments can be performed to elucidate the nature of the stimulus. To eliminate the possibility that the arrestment response is due to some physical property of the patch (e.g., the texture of the wax secretions left by mealybugs, or depressions caused by feeding larvae), one can attempt to dissolve the putative kairomone either in distilled water, hexane or another suitable solvent, and then apply the solution to a surface, for example a leaf or a glass plate, which has never borne host larvae. If an arrestment response is still observed, it can be concluded that the soluble substance is a kairomone. For a detailed experimental study of the arrestment response in a parasitoid, conducted along these lines, see Waage (1978) and Fig. 1.12.

Kairomones provide quantitative, in addition to qualitative information. Several parasitoid species, when presented with several patches of kairomone in different concentrations, have been shown to spend longer periods searching those patches with the higher kairomone concentrations than the patches with the lower concentrations, at least over part of the range of concentrations (Waage, 1978; Galis & van Alphen, 1981; Budenberg, 1990; Hare & Morgan, 2000). Because kairomone concentration varies with host density, parasitoids can obtain information concerning the profitability of a patch, even before they encounter hosts. Honeydew produced by the aphid *Brevicoryne brassicae* provides not only a qualitative cue in host location, but also is a source of information on the density of hosts within a patch for the parasitoid *Diaeretiella rapae* (Shaltiel & Ayal, 1998).

1.6.4 Responses to Parasitoid Odours and Patch Marks

Parasitoid Odours

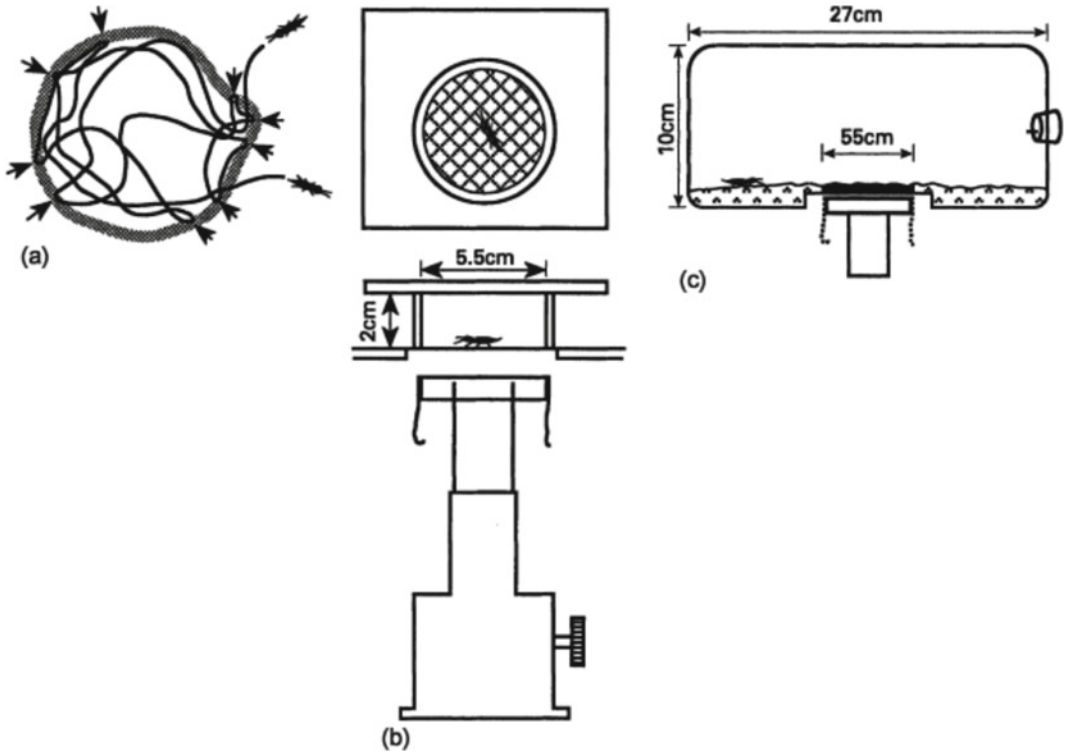
Janssen et al. (1991) showed, using olfactometer experiments, that *Leptopilina heterotoma* is attracted to the odour of stinkhorn fungi containing larvae of *Drosophila phalerata*. When these patches are offered in an olfactometer together with similar patches on which searching females

of *L. clavipes* are present, *L. heterotoma* avoids the odour fields of patches containing *L. clavipes* females. The conclusion from these observations is that *L. clavipes* produces an odour whilst searching, which repels its competitor *L. heterotoma*, at least when the latter is presented with the choice between host-containing patches emitting this odour and host-containing patches that lack the odour. Price (1981) suggests that the function of the strong odour emitted by some female ichneumonids, which may be noticed when these insects are handled, likewise signals the insects' presence to other parasitoids. Furthermore, kairomones combined with odours from conspecifics may help parasitoids avoid intraspecific competition. *Venturia canescens* will normally orientate towards host kairomones but will avoid the odour plumes which contain both host kairomones and the odour of conspecific females (Castelo et al., 2003).

Höller et al. (1991) found evidence that foraging primary parasitoids of aphids are repelled by odours produced by adult hyperparasitoids. Furthermore, individuals of the aphid *Sitobion avenae* that have been attacked by a primary parasitoid, *Aphidius ervi*, show differential responses to odours released by a hyperparasitoid, *Alloxysta victrix*. At 120 h after attack, the aphids are attracted to the volatiles, yet at 160 h after attack they are repelled by the same cue. Since unattacked aphids show no responses to these cues, Guerra et al. (1998) suggest that, as behavioural control passes from aphid to parasitoid over time, the adaptive benefits of responding to these cues will also change.

In cases where the odour of a parasitoid repels conspecifics, the substance is a pheromone, whereas in cases where heterospecific competitors are repelled, there is some justification in describing the substance as an allomone. However, because of similar problems to those mentioned below when discussing patch marking, the use of the term allomone should be avoided here.

It is not known how widespread the use of repellent odours is among insect parasitoids,



◀ **Fig. 1.12** Arrestment and patch time allocation in parasitoids: Waage's (1978) classic study of *Venturia canescens*. **a** An experiment carried out to investigate arrestment behaviour of *V. canescens* in response to contact with a kairomone. The path of a walking female was observed on a glass plate, upon which 1 ml of ether extract of ten pairs of host (the lepidopteran *Plodia interpunctella*) manidibular glands had been placed and allowed to evaporate. Stippling denotes the edge of the patch. Upon encountering the patch edge from the outside, a female stops and begins to apply the tips of its antennae rapidly upon the substratum. It then proceeds onto the patch at a reduced walking speed (inverse orthokinesis). Within the patch, the wasp occasionally stops walking and probes the substratum with its ovipositor. When the wasp encounters the patch edge from within the patch, it turns sharply away from the edge. The wasp eventually leaves the patch, presumably due to waning of the arrestment response, e.g., through habituation or sensory adaptation to the chemical stimulus. **b** Apparatus used to test the hypothesis that the patch edge response of *V. canescens* is to the removal of the chemical stimulus, and not to the patch edge *per se*. The terylene gauze screen was impregnated with host mandibular secretion by confining ten fifth instar host larvae between two sheets of gauze. The lower sheet was then stretched over a Petri dish, as in the figure. By raising and rapidly lowering the contaminated screen, Waage (1978) could precisely control when a wasp (in the upper chamber) was 'on' and 'off' the patch. A wasp's movements were traced with a felt-tipped pen on the plate glass roof of the chamber (nowadays this could be done using video recording coupled with analysis of movements using computer software, Sect. 1.4). Over the first centimetre travelled following stimulus removal, most wasps made a reverse turn, which may be considered to be a klinotactic (i.e., directed) response because the turn oriented the wasps back towards the the point from where the stimulus was removed. Thus, Waage (1978) concluded that the patch edge response of *V. canescens* is due to the removal of the chemical stimulus, not to the patch edge *per se*, i.e., his hypothesis was supported. **c** Apparatus used by Waage to test the effect of kairomone concentration on patch residence time. 'Patches' were made by confining different numbers of host larvae, together with food medium, between terylene gauze sheets for several hours. The larvae were then removed. For each kairomone concentration, the contaminated patch of food medium (minus the hosts) was held over the central part of the floor of the chamber (blackened area). An empty Petri dish was raised beneath the patch (see next experiment). Two arbitrary time intervals (14 s and 60 s continuously off a patch) were used as criteria for determining patch leaving by wasps. Application of either of these criteria indicated that the duration of the first visit to a patch increased markedly with increasing kairomone concentration. The apparatus was also used to test the effect of ovipositions on patch residence time. A patch of host-contaminated food medium was stretched over the central part of the chamber floor, and at the onset of the experiment a dish containing 30 host larvae was raised beneath the patch. Each wasp was allowed to make an oviposition into a host as soon as she entered the patch. During the resting period following that oviposition, the dish containing host larvae was replaced with an empty one. Oviposition was found to produce a marked increase in the duration of the first patch visit by a wasp. Another experiment was carried out by Waage (1978), which demonstrated that oviposition does not elicit a significant arrestment response in the absence of the kairomone. This experiment employed apparatus (**b**). A host-contaminated terylene gauze screen, with or without host larvae beneath it, was raised beneath the chamber. A single wasp was exposed either to the chemical stimulus alone for the duration of one bout of probing, or to the chemical stimulus with hosts present for one oviposition of similar duration. The screen was then lowered, so removing the kairomone stimulus, and the time taken for the wasp to leave the chamber floor and then climb onto one of the chamber sides was recorded (this behaviour being interpreted as the cessation of any response elicited by the contact chemical). No significant difference in the amount of time taken to abandon the host area was observed between the treatments with oviposition and those without. From Waage (1978), reproduced by permission of Blackwell Publishing

largely because this issue has not been studied in a systematic way. Like other infochemicals used by parasitoids, odours produced by adult parasitoids can potentially have a profound effect on patch choice and time allocation by individual wasps and thus on the distribution of parasitoids over a host population.

Patch Marking

Some parasitoid species are known to leave chemical marks on surfaces they have searched (Galis & van Alphen, 1981; Sheehan et al., 1993; Couchoux & van Nouhuys, 2014). This marking behaviour can have a number of functions. By leaving a scent mark on the substratum, a parasitoid can avoid wasting time and energy in searching already visited areas. A female can also use the frequency with which she encounters marks to determine how well she has searched the patch, and so assist in the decision when to leave the patch. When encountered by conspecific or heterospecific competitors, marks sometimes induce the competitor to leave an area. *Pleolophus basizonus*, *Orgilus lepidus*,

Asobara tabida, *Microplitis croceipes*, *Halticoptera rosae*, *H. laevigata* and *Hyposoter horticola* (Price, 1970; Greany & Oatman, 1972; Galis & van Alphen, 1981; Sheehan et al., 1993; Hoffmeister, 2000; Hoffmeister & Gienapp, 2001; Couchoux & van Nouhuys, 2014) mark areas they search, and females spend less time in areas previously searched by conspecifics. In the case of a heterospecific competitor, the marker substance could be termed an allomone. However, leaving the patch may not always be in the interest of the competitor; the competitor may stay and superparasitise the hosts parasitised by the first female (Sect. 1.9.4). Thus, the use of the term allomone should be avoided in this context.

The use of patch-marker substances can be demonstrated by offering patches containing kairomone, but not hosts, to a parasitoid. After the parasitoid has left the patch, a second parasitoid is introduced on to the same patch. If the second insect stays on the patch for a shorter period than the first, the existence of a mark left by the first has, given sufficient experimental replication, been demonstrated.

Predators may also patch-mark. Nakashima et al. (2002) showed that the insect predator *Orius sauteri* avoides patches where they have recently foraged, although this behaviour is not exhibited when the predator has not recently fed. The patch marks appear to be relatively short lived (<1 h) and may simply prevent the females from foraging in an area previously searched.

It is not only insect natural enemies that respond to such cues. The prey themselves may also respond to odour cues left by foraging predators or parasitoids. For example, spider mites (*Tetranychus urticae*) will avoid foraging in patches that have previously held predators, and this avoidance is greater if the predators have been feeding on *T. urticae* (Grostal & Dicke, 2000). Most studies of predator and parasitoid foraging behaviour assume that such avoidance does not take place.

1.6.5 Search Modes Within a Patch

While kairomones and other cues can arrest parasitoids and predators in host/prey patches and so increase the probability of encounter, host/prey location is itself likely to be in response to non-chemical, e.g., visual and tactile cues. For example, in coccinellid predators, prey honeydew acts as an arrestant stimulus for adults (van den Meiracker et al., 1990: *Diomus* sp., *Exochomus* sp.; Heidari & Copland, 1993: *Cryptolaemus montrouzieri*), but the prey are located in response to visual cues (Stubbs, 1980: *Coccinella septempunctata*; Heidari & Copland, 1992: *C. montrouzieri*). Stubbs (1980) devised a method for calculating the distance over which prey are detected (see also Heidari & Copland, 1992). Another method was designed to calculate the distance over which insect parasitoids detect their hosts (Bruins et al., 1994). In the coccinellid *Coccinella septempunctata*, honeydew acts as arrestant stimulus that increased exploitation of prey patches by larvae, but location of the prey occurs only upon physical contact (Carter & Dixon, 1984).

It has been shown for a number of predators that arrestment occurs as a consequence of prey capture (Dixon, 1959; Marks, 1977; Nakamuta, 1982; Murakami & Tsubaki, 1984; Ettifouri & Ferran, 1993). In this way, the insect's searching activities are concentrated in the immediate vicinity of the previously captured prey, increasing the probability of locating another prey individual. The adaptive value of such behaviour for predators and parasitoids of insects that have a clumped distribution, such as aphids, is obvious. Predators also show arrestment after capturing a prey individual but failing to feed on it, even a failed encounter with prey is an indication that a clump of prey has been found (Carter & Dixon, 1984). Carter and Dixon (1984) argued that the latter behaviour is particularly important for early instars of coccinellids, since the prey capture efficiency of these instars is

relatively low. In final instar larvae of the coccinellid *Harmonia axyridis*, arrestment in response to prey capture occurs only if the predators are provided with the same prey species as they were reared upon, indicating a strong conditioning effect (Ettifouri & Ferran, 1993). Arrestment of the aboreal ponerine ant *Platythyrea modesta* is affected by prey size. Small prey required contact, whereas larger prey, such as grasshoppers, elicit arrestment at a distance (Djieto-Lordon et al., 2001). Following arrestment, the ants attacked without antennation. Small prey species are killed using pressure from the mandibles, whereas larger prey are stung.

Arrestment in the above cases can be studied in the same way as arrestment of natural enemies in response to kairomones, i.e., by analysing the search paths of predators and parasitoids and by measuring the proportion of the total time spent searching designated unit areas within an arena.

Species of parasitoid attacking the same hosts may differ in the way they search a patch. In parasitoids of concealed anthomyiid, calliphorid, drosophilid, muscid, phorid, sarcophagid and sepsid fly larvae, at least three different search modes exist (Vet & van Alphen, 1985). Wasps may either: (1) probe the microhabitat with their ovipositors until they contact a host larva (ovipositor search); (2) perceive vibrations in the microhabitat caused by movements of the host and use these cues to orient themselves to the host (vibrotaxis) which is then probed with the ovipositor; or (3) drum, with their antennae, the surface of the microhabitat until they contact a host (antennal search).

To determine which search mode a parasitoid species uses is easy in the case of ovipositor search or antennal search, where brief observation of a searching female suffices to classify her search mode. However, it can be difficult to prove that vibrotaxis occurs, because of the possibility that the parasitoid locates its hosts by reacting to a gradient in kairomone concentration or some other chemical cue, or to infrared radiation from the host. Therefore, we will consider this search mode in more detail.

Parasitoids have been shown to respond to vibratory stimuli issuing from foraging hosts when they are searching for potential victims. Meyhöfer et al. (1994, 1997) found that the leafminer *Phyllonorycter malella* produces vibrations while feeding, and that the parasitoid *Sympiesis sericeicornis* responds to these cues by increased rates of turning in the vicinity (vibrokinesis). There is also some evidence for vibrotaxis, but this is more circumstantial. For example, *Asobara tabida* and *Leptopilina longipes*, two common parasitoids of *Drosophila* species, will fail to locate immobilised hosts (van Alphen & Drijver, 1982; van Dijken & van Alphen, 1998). Indeed, it has been suggested that the rover/sitter polymorphism in larval *Drosophila melanogaster* (Alwash et al., 2021) may be maintained by frequency-dependent selection resulting from the relative proportions of vibrotactic parasitoids within the community of larval parasitoids (Osborne et al., 1997; see also Hodges et al., 2013).

In a valuable review, Meyhöfer and Casas (1999), however, pointed out some pitfalls in the study of the use of vibratory stimuli by parasitoids searching for hosts. Many of these are associated with experimental design, where the use of immobilised larvae (e.g., by freezing, dipping in hot water, needle insertion) introduces the confounding factors associated with reduced metabolic rate (influencing heat or CO_2 output) and changes in the chemical cues emanating from potential hosts. Unless these confounding factors are controlled for, it is difficult to confirm that changes in parasitoid behaviour are the result of responses to vibratory cues. A second issue they raised is the need to confirm that the host does indeed produce vibratory cues to which the parasitoid can and does respond. Very few studies satisfactorily deal with these issues, although techniques such as laser vibrometry are available to characterise these vibrational signals (Meyhöfer et al., 1994).

Wäckers et al. (1998) used laser vibrometry to infer the ability of the pupal parasitoid *Pimpla turionellae* to locate potential hosts. Clearly, the host pupae themselves do not produce vibrations; instead, the parasitoid itself appears to generate vibrations that can then be used, in a manner analogous to sonar, to locate hosts. The

technique used by Wäckers et al. (1998) was particularly ingenious: by using paper cylinders of differing thickness and a cigarette filter to serve as a 'host', the authors were able to show that, as the thickness of the substrate increased, the number of oviposition attempts decreased. They suggest that the parasitoid responded to differences in resonance between hollow and solid sections of substrate, and that increasing thickness of paper reduced the ability of the parasitoid to distinguish between sections.

Vibrations may also be used by potential hosts as a warning that a parasitoid may be about to attack. Bacher et al. (1997), again using laser vibrometry, showed that the late instar larvae and pupae of the leafminer *Phyllonorycter malella* reacted defensively to certain frequencies produced by oviposition insertion by the parasitoid *Sympiesis sericeicornis*. Such ability to avoid attack may prove to be common among leafminers. Using the same system, Djemai et al. (2001) used artificial vibrations matched to the frequencies resulting from *Sympiesis sericeicornis* attack, and this elicited the same defensive behaviours in the host. This provides excellent empirical support for the conclusions drawn by the earlier study.

The reason why it is important to determine the search mode of a parasitoid or a predator is that different search modes lead to different encounter rates with hosts in the same situation. Thus, a parasitoid using vibrotaxis as a search mode may be more successful in finding hosts when the hosts occur at low densities, while ovipositor search may be more profitable at high host densities. Antennal search results in encounters with larvae on the surface, while ovipositor search can also result in encounters with hosts buried in the host's food medium. However, Broad and Quicke (2000) showed that the use of vibrotaxis is positively correlated with host depth in the substrate, controlling for parasitoid size. This suggests that in substrates where ovipositor searching is time-consuming (e.g., where the host is relatively deep in the substrate), vibrotaxis may be more common than the aforementioned argument suggests. Often, the searching behaviour of a parasitoid comprises a combination of search modes, as the insect responds to different cues while locating a host. It is therefore not always possible to place the behaviour of a parasitoid in one category.

Predators may employ a combination of search modes. The larvae of the predatory water beetle *Dytiscus verticalis* may either behave as sit-and-wait predators when prey density is high, or hunt actively for prey when prey density is low (Formanowicz, 1982). Such variety is common, and many species that are traditionally considered to be ambush predators (e.g., mantids, see below) frequently actively search for prey.

Pit-dwelling antlion (*Myrmeleon* spp.) larvae provide the classic example of an ambush predator. The larvae excavate funnel-shaped holes in loose sand, and it is the latter that prevents potential prey from escaping. The spatial distribution of the antlion *Myrmeleon immaculatus* reflects that of prey density, minimising the need to move to a new pit location (Crowley & Linton, 1999). The antlion larva waits at the base of the pit, with only its relatively large mandibles projecting from the sand. Once a victim becomes trapped, the larva suddenly grabs its prey and drags it under the sand. This has the advantage of rendering physical defences, such as biting or formic acid, useless (New, 1991). Given that the ambush strategy is risky (i.e., the presence of food is unpredictable) and that manufacturing and maintaining the pit is costly (Lucas, 1985; Hauber, 1999), it is unsurprising that antlions have relatively low metabolic rates (van Zyl et al., 1997). Larvae can survive for relatively long periods without food, albeit at the cost of a long development period.

In contrast to situations where camouflage is critically important, some 'sit-and-wait' predators employ conspicuous colouration, e.g., several species of orb-web spider. The spiny spider, *Gasteracantha fornicata*, has a strikingly coloured yellow-and-black-striped dorsal surface. Spiders which were dyed black captured fewer prey individuals, supporting the hypothesis that bright colours helped attract visually orienting prey (Hauber, 2002).

1.6.6 Host Recognition by Parasitoids

Generally, specific (although not necessarily host species-specific) host-associated stimuli need to be present for triggering of oviposition behaviour by parasitoids following location of a prospective host. The role these stimuli play in host recognition has been investigated mainly by means of very simple experiments.

For many parasitoids, host size appears to be important for host recognition. In a classic experiment, Salt (1958) presented female *Trichogramma* with a small globule of mercury – smaller than a host egg—and observed that the parasitoid did not respond to the globule. However, Salt (1958) then added minute quantities of mercury to the globule, whereupon a female would mount it, examine it and attempt to pierce it with her ovipositor. When Salt (1958) continued adding quantities of mercury to the globule, a globule size was reached where a wasp again did not recognise it as a prospective host.

Host shape can be important in host recognition. A number of workers have placed inanimate objects of various kinds inside either hosts or host cuticles from which the host's body contents have been removed and have shown that some host shapes are more acceptable than others.

One needs to be cautious in interpreting the results of experiments where hosts or host dummies of various sizes and shapes are presented to parasitoids. If a parasitoid is found to attempt oviposition more often in large dummies than in small ones, or in rounded dummies than in flattened ones, the stimuli involved could be visual, tactile or both. Some investigators have failed to determine precisely which of these stimuli are important (but see Bruins et al., 1994). Similar caution needs to be applied to experiments in which dummies of different textures are presented to parasitoids.

As can often be inferred from direct observations on the behavioural interactions of parasitoids and hosts, movement by the host can be important in triggering oviposition behaviour. A simple experiment for investigating the role of host movement in host recognition involves killing hosts, attaching them to cotton or nylon threads, moving both these and similarly attached living hosts before parasitoids, and determining the relative extent to which the dead and living hosts are examined, stabbed, drilled or even oviposited in by the parasitoids.

Kairomones play a very important (although not necessarily exclusive) role in host recognition by parasitoids. In an elegant series of experiments, Strand and Vinson (1982) showed how, if glass beads the size of host eggs are uniformly coated with material present in accessory glands of the female host (host eggs normally bear secretions from these glands) and are presented to females of *Telenomus heliothidis* (Scelionidae), the insects will readily attempt to drill the beads with their ovipositors. Female parasitoids, when presented with either clean glass beads or host eggs that had been washed in certain chemicals, were, on the whole, unresponsive. Strand and Vinson (1983) analysed the host accessory gland material and isolated proteins from it (by electrophoresis); two proteins were shown to be particularly effective in eliciting drilling of glass beads. It cannot be assumed from these findings that *T. heliothidis* will recognise any object that is coated in kairomone as a host: host size and shape are also important criteria for host acceptance. Similar findings have been reported for several other species (e.g., Conti et al., 2003: *Trissolcus brochymenae*; Takasu et al., 2003: *Ixodiphagus hookeri*). In some cases, the active compound has been identified: O-caffeoylserine, produced by the cassava mealybug, elicits host-acceptance behaviour in the encyrtid parasitoids *Acerophagus coccois* and *Aenasius vexans* (Calatayud et al., 2001).

Weinbrenner and Völkl (2002) took a different approach to understanding the importance of contact kairomones in host recognition by *Aphidius ervi*. Wet pea aphids were not accepted as hosts, which the authors suggest resulted from the parasitoids being unable to detect the host's kairomones. Another useful approach to studying the role of kairomones would be to take a polyphagous parasitoid species and determine whether the recognition kairomone is different or the same for each of its host species. Van Alphen and Vet (1986) showed that the braconid

parasitoid *Asobara tabida* discriminates between the kairomone produced by *Drosophila melanogaster* and that produced by *D. subobscura*. Acceptance of a prospective host for oviposition also depends upon whether the host is already parasitised. This important aspect of parasitoid behaviour is dealt with later in Sect. 1.9.

1.6.7 Host and Prey Selection

Host Species Selection

Many parasitoid species are either polyphagous or oligophagous. Strictly monophagous species are relatively uncommon. When different potential host species occur in different habitats, a parasitoid 'decides' which host species is to be attacked by virtue of its choice of habitat in which to search. Sometimes, potential host species can be found coexisting in the same patch (e.g., two aphid species living on the same host plant, larvae of different fly species feeding in the same corpse, etc.). In these cases, experiments on host species selection are relevant, and can demonstrate whether the parasitoid has a preference for either of the species involved. Preference is defined as follows: a parasitoid or predator shows a preference for a particular host/prey type when the proportion of that type oviposited in or eaten is higher than the proportion available in the environment. This is the traditional 'black box' definition (Taylor, 1984), so called because it does not specify the behavioural mechanisms involved. For example, a parasitoid may encounter a host individual and accept it, but the host may then escape before the parasitoid has an opportunity to oviposit (likewise, prey may escape from a predator following acceptance). If host types differ in their ability to escape, they will be parasitised to differing extents even though they may be accepted at the same rate. Conversely, they may be accepted at different rates but be parasitised to the same extent. It could be argued that preference, to be more meaningful behaviourally, ought to be defined in terms of the proportion of hosts or prey accepted. However, it may not be possible in experiments to observe and score the number of acceptances (one reason being that the insects do not display obvious acceptance behaviour).

Often experiments designed to test for a preference score the number of hosts parasitised, or prey fed upon, after a certain period of exposure where equal numbers of each species have been offered (Sect. 1.12 describes a different approach). There is, however, a problem with this approach: the number of hosts oviposited in, or prey eaten, depends on the number of encounters with individuals of each species, and the decision to oviposit, or feed, on the less preferred species may be influenced by how often the female has the opportunity to oviposit, or feed, on the preferred species. Encounter rates (Sect. 1.7) may also be unequal for two host, or prey, species, due to factors such as differences in size or activity. Therefore, species selection should preferably be investigated in such a way that encounter rates with both species are equal. This requires pilot experiments, with equal numbers of each species offered simultaneously, to calculate the ratio in which both types should be presented so as to equalise encounter rates.

Mathematical formulae used for quantifying preference (whether for species or for stages) are many and varied (Chesson, 1978, 1983; Cock, 1978; Settle & Wilson, 1990), but the most widely used measure of preference is the following (Sherratt & Harvey, 1993):

$$\frac{E_1}{E_2} = c\frac{N_1}{N_2} \tag{1.1}$$

where N_1 and N_2 represent the numbers of two host, or prey, types available in the environment, and E_1 and E_2 represent the numbers of the two host, or prey, types oviposited in or eaten. The parameter c is the preference index and can be viewed as a combined measure of preference and encounter probability (Sect. 1.12). A value of c between zero and one indicates a preference for host, or prey, type 2, whereas a value of c between one and infinity indicates a preference for host, or prey, type 1. Mathematical formulae used in testing whether preference varies with the

relative abundance of the different host or prey, types are discussed in Sect. 1.12.

A rather more sophisticated approach has been suggested by Sakuma (1998), using probit analysis. This method overcomes the problems associated with standard probit analysis (an all-or-nothing approach), taking into account differences in the strength of the stimulus (e.g., number of hosts or quantity of odour cues). The program (available from Masayuki Sakuma, Graduate School of Agriculture, Kyoto University, Kyoto 606–8502, Japan), involves a regression of the probit-transformed number of responses against the log of the dose (or here, number of hosts). Such an approach would be suitable also for analysing preference data from olfactometer experiments.

Optimal host selection models predict that the acceptance of a less profitable host species depends on the encounter rate with the more profitable host species. The less profitable species should always be ignored if the encounter rate with the more profitable species is above some threshold value but should be attacked if the encounter rate with the more profitable species is below that threshold value (Charnov, 1976; Stephens & Krebs, 1986). Note that if recognition of prey is not instantaneous, then acceptance of the less profitable host species depends on the encounter rates with both of the host species and on the time taken for recognition to take place. Often, for the convenience of the researcher, relatively high densities of hosts, resulting in high encounter rates, are offered in laboratory experiments. This will produce a bias towards more selective behaviour. For example, in laboratory experiments with high encounter rates, the *Drosophila* parasitoid *Asobara tabida* is selective when offered the choice between two host species differing in survival probability for its offspring (van Alphen & Janssen, 1982) and avoids superparasitism (van Alphen & Nell, 1982). However, in the field, when encounter rates are equal to or lower than one host per hour, wasps always generalise and superparasitise (Janssen, 1989). If one is interested in knowing the performance of a parasitoid species in the field, where host densities are often very low, in the laboratory one should use host densities equivalent to those occurring in the field. The high densities often offered in the laboratory may allow the researcher to obtain much data over a short period of observation but the insect's behaviour in such experiments may not be representative of what happens in the field.

To understand the adaptive significance of host preferences, the relative profitability of different host species can be assessed, in the first instance, by recording the survival rates of parasitoid progeny in the different hosts. Even if no differences in the probability of parasitoid offspring survival are recorded, one cannot automatically assume that the host species concerned are equally profitable. Handling times may vary with host species, as may the fecundity and other components of the fitness of parasitoid progeny, and ideally, these should be measured.

Experiments on prey choice by predators are influenced by prey densities offered in a manner similar to that described above for parasitoids. Because searching activity is influenced by the amount of food in the gut (more precisely, the degree of satiation), a predator's feeding history may determine the outcome of experiments on prey choice (Griffiths, 1982; Sabelis, 1990; but this may not always be the case, e.g., see de Kraker et al., 2001).

So far, we have considered innate host and prey preferences. Preferences may change with experience (Sect. 1.12) Preferences may also change with the physiological state of the predator or parasitoid. For example, Sadeghi and Gilbert (1999, 2000) found that the hoverflies *Episyrphus balteatus* and *Syrphus ribesii* both preferentially attacked pea and rose aphids over nettle aphids, and that the strength of this relationship weakened with time. This is likely due to the influences of host deprivation and egg load on oviposition rates differeing between the species.

One crucial, yet almost completely ignored, factor in parasitoid host choice behaviour concerns the presence of genetic variation within a given population. Without this variation, populations will not be able to evolve in response to changes in the host community. Genetic variation

explains the variation among parasitoid individuals in host preference. Rolff and Kraaijeveld (2001) found that virulent lines of the parasitoid *Asobara tabida* were more likely to accept *Drosophila melanogaster*, a host species with a strong immune response, than control lines which preferentially attacked the non-resistant species, *Drosophila subobscura*. Host species selection is further discussed in Sect. 1.12.

Host Stage Selection

Parasitoids may encounter different developmental stages of the host within a patch. Those stages potentially vulnerable to attack may differ in their profitability. For idiobionts (parasitoids in which the host does not grow beyond the stage attacked and which therefore is a fixed 'parcel' of resource, Fig. 1.13a), small host stages may provide inadequate amounts of resource to permit the successful development of offspring. Even where successful development of idiobiont progeny is possible in small hosts, the resultant offspring are small and therefore oviposition constitutes less of a fitness gain (in parasitoids, body size can determine components of fitness, such as fecundity, longevity, searching efficiency

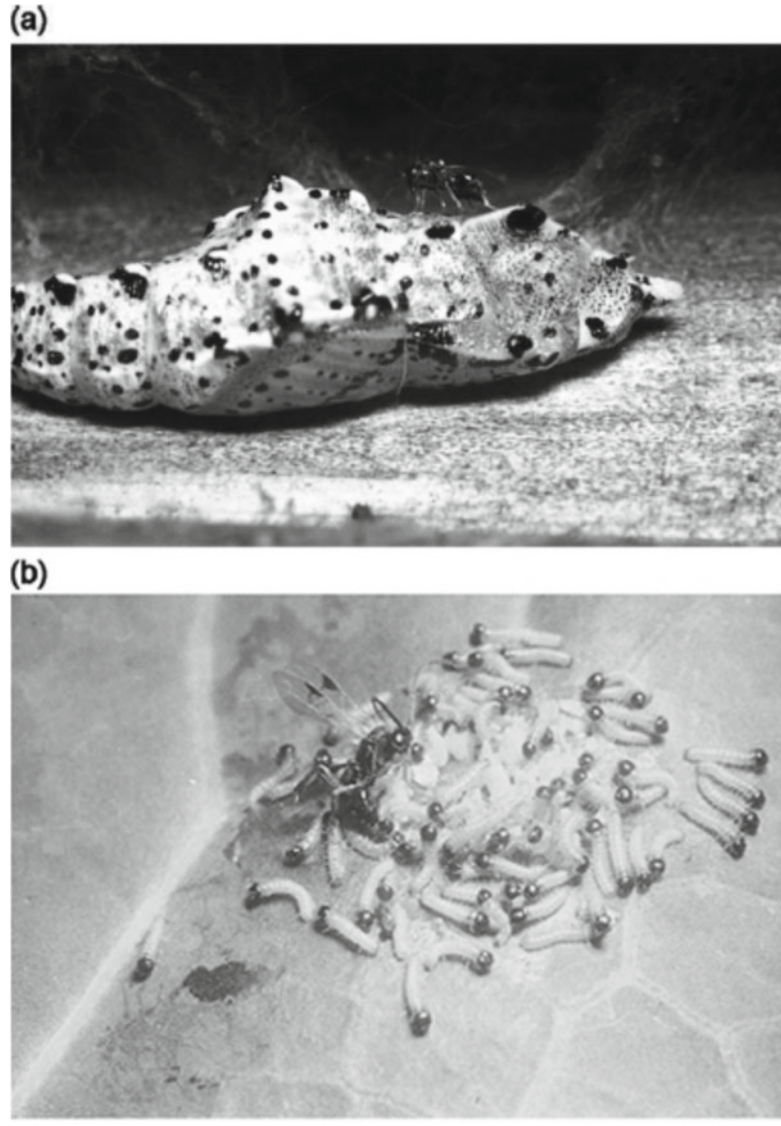


Fig. 1.13 Idiobiont and koinobiont parasitoids (both gregarious) of the same host species, *Pieris brassicae* (Lepidoptera: Pieridae): **a** *Pteromalus puparum* (Pteromalidae) ovipositing into host's pupal stage. For the parasitoid's progeny, the pupa is a 'fixed' parcel of resource, as it is a non-feeding, non-growing stage. This parasitoid is therefore an idiobiont. **b** *Cotesia glomerata* (Braconidae) ovipositing into newly hatched host larvae which will continue to feed, grow and develop during parasitoid development. This parasitoid is therefore a koinobiont. *Source* Premaphotos, UK

and competitive ability; Hardy et al., 1992b, 2013; Visser, 1994; Guerra-Grenier et al., 2020). Although they exploit a growing amount of host resource, koinobionts (parasitoids that allow their hosts to continue to feed and develop, Fig. 1.13b) also may display a positive relationship between adult body size and host size, although the relationship may not be linear (Sequeira & Mackauer, 1992; Harvey et al., 1994, 1999).

For both idiobionts and koinobionts, smaller hosts may require less time for handling and represent less of a risk of injury resulting from the defensive behaviour of the host (Sect. 1.20.3). For koinobionts (most of which are endoparasitoids), small hosts may present parasitoid progeny with lower mortality risk from encapsulation (van Alphen & Drijver, 1982; Sagarra & Vincent, 1999; Sect. 2.10). Females of both idiobionts and koinobionts may also gain in fitness from ovipositing in, or on, older larvae, owing to the fact that, under field conditions, host mortality resulting from predation and/or intraspecific competition is more severe in early host stages than in late ones (Price, 1975). Thus, it is often the case that parasitoids prefer to attack certain host stages and even avoid or reject other stages for oviposition. Nevertheless, for several idiobiont parasitoids of fly pupae, there is evidence that older hosts are not preferred, since these provide fewer resources for their developing offspring (e.g., King, 1998).

The distribution of hosts of different size over the host plant may influence encounter rates and thus host stage selection behaviour. Later instars of mealybugs are often surrounded by earlier instars. Encounter rates with younger instars may be higher, and those with larger ones lower than predicted, based on their densities. Young larvae of calliphorid and drosophilid fly species feed near the surface of the substrate, while older larvae may burrow deeper, possibly out of the reach of parasitoids.

Often, hosts are not passive victims of their parasitoids (Sect. 1.20.3). Behavioural defences of hosts (which are often more effective in later host stages) can cause a problem of data interpretation. Should encounters that do not result in parasitism of the host be scored as acceptances or as rejections? If the parasitoid clearly displays behaviour that is normally associated with host acceptance, such as the turning and stinging shown by encyrtids (Fig. 1.14), the encounter should be classified as an acceptance.

The ability of late-stage hosts to defend themselves from attack better than early-stage hosts may account for a host stage preference. The cost in terms of lost 'opportunity time' (time that could be spent in more profitable behaviour) when attacks on late-stage hosts fail, may outweigh the fitness gain per egg laid (Kouame & Mackauer, 1991). Defence against parasitoids may also incorporate an immune response. It is generally the case that the risk of encapsulation is higher in later compared with earlier host larval instars (Sect. 2.10). This suggests that foraging endoparasitoids should preferentially attack host stages with a weaker immune response, whereas ectoparasitoids, which are not exposed in a similar manner to the host's immune response, should show a host stage-based preference.

Host size selection by parasitoids is not limited to the decision of whether to oviposit or reject the host. It also involves the decision of which sex the offspring ought to be, and, for gregarious parasitoids, how many eggs to lay

Fig. 1.14 Host-acceptance behaviour in the encyrtid parasitoid *Apoanagyrus lopezi*: The female examines the host with its antennae. Acceptance is indicated by the wasp turning towards the host to insert its ovipositor. Sometimes, the host escapes whilst the wasp is turning—acceptance therefore does not necessarily lead to oviposition

(Fig. 1.1). For practical reasons, we analyse those decisions as isolated steps, but one should bear in mind that they are interrelated, and that it is wise to study host size selection in combination with clutch size and sex allocation decisions. Host size selection in relation to clutch size is discussed further in Sect. 1.10.

Predators are usually less specific in their choice of prey than parasitoids, although some predators show a preference for larger prey or certain instars (Cock, 1978; Thompson, 1978; Griffiths, 1982). Prey size selection in predators may also change with the size of the predator (Griffiths, 1982).

Host selection decisions by one female may alter over time during an experiment because these decisions are affected by experience, egg load and stochastic variation in encounter rates. Such changes in decisions are one of the reasons why partial preferences are always found instead of the absolute, i.e., all-or-none preferences predicted by static prey choice models. If one is interested in questions such as how egg load should influence host selection, one should construct dynamic optimisation models as described by Mangel and Clark (1988; see Heimpel et al., 1998, for an example).

1.7 Measuring Encounter Rates

The encounter rate of individual parasitoids with hosts is an important parameter in many optimality models. Because not every encounter will be followed by oviposition, and because not every oviposition will be in an unparasitised host, encounter rate is not equal to the number of hosts parasitised per time unit. Encounter rates can be used to calculate predicted rates of offspring deposited per time unit with a particular optimal foraging model.

Optimality models divide the time budget of a foraging animal into searching time, recognition time and handling time. Encounter rate is expressed and measured as the number of encounters per unit of searching time, thereby excluding recognition time and handling time. Because encounter rates are not always a linear function of host density, it is necessary to measure them at a range of host densities.

To measure encounter rates, observe a female parasitoid continuously during some time period and make a complete record of her behaviour. From this record, the number of encounters and the net period of time spent searching can be calculated. The encounter rate of a parasitoid searching a patch containing a number of hosts may not be constant over the foraging period for the following reasons:

1. Parasitised hosts are encountered at a lower rate and the number of parasitised hosts increases during the observation period. A lower encounter rate with parasitised hosts may occur because hosts are paralysed by the wasp, and so move less (van Alphen & Galis, 1983).
2. The search effort of the wasp decreases, either in response to contact with its own marker substance (Sect. 1.6.4) or because its supply of mature eggs dwindles.

One method of eliminating some of the causes of decreased encounter rate is to replace each parasitised host with an unparasitised one during the course of an experiment. This is not always possible, e.g., sessile hosts such as scale insects cannot easily be removed and replaced. Replacing parasitised hosts may also affect encounter rate: it may disturb the searching wasp, and so decrease encounter rate, or it may increase encounter rate when the parasitoid is of a species that reacts to host movements and the freshly introduced hosts move more than those already present. Finally, a parasitoid may learn, during the experiment, that the observer is introducing better-quality hosts and simply walk towards the forceps or paint brush used to introduce the new host, as has often been observed with alysiine braconid parasitoids. Therefore, when measuring encounter rates, one should not replace parasitised hosts but instead keep the period of observation short, in order to avoid accumulation of parasitised hosts and marker substance.

Measuring encounter rates using a single-patch experimental design will overestimate the encounter rates that would be recorded in a

multi-patch, i.e., more natural, environment, because the time spent in inter-patch travel is not accounted for. Since it is often difficult, or impossible, to measure inter-patch travel times, the simplest approach is to measure, over a fixed period, the attack rate of a known number of parasitoids foraging in a spatially heterogeneous environment (Waage, 1979; Hassell, 1982).

1.8 Host Feeding

The females of many synovigenic parasitoids (Sects. 1.16.2 and 2.3.4) not only parasitise hosts but also feed on them (Jervis & Kidd, 1986, 1999; Heimpel & Collier, 1996; Jervis, 1998 Ueno, 1998a, 1998b, 1999a, 1999b, 1999c; Yang et al., 2012; Abram et al., 2019; Zhang et al., 2019; Miksanek & Heimpel, 2020; Cusumano et al., 2022a, 2022b). Host feeding supplies the females with materials for continued egg production and for somatic maintenance (Bartlett, 1964; Jervis & Kidd, 1986, 1999; Pérez-Lachaud & Hardy, 1999). Giron et al. (2002) showed that the parasitoid *Eupelmus vuilletti* host fed upon the host's haemolymph. The haemolymph is rich in proteins and various sugars, and it is these sugars that are responsible for the increased longevity of *E. vuilletti*. In some parasitoid species, host feeding causes the host to die (so-called 'destructive' host feeding), rendering it unsuitable for oviposition. Even with those species that remove small quantities of host materials such that the host survives feeding ('non-destructive' host feeding), the nutritional value of the host for parasitoid offspring may, as a result of feeding, be reduced and the female may lay fewer (gregarious species), or no eggs in it. For example, lepidopteran hosts previously host-fed upon by *Pimpla nipponica* produced fewer and smaller wasps when subsequently parasitised (Ueno, 1997). Thus, while host feeding potentially increases future fitness via subsequently increased egg production, the fitness gain is at the cost of current reproduction.

Most authors have supposed that host feeding has a short-term effect on parasitoid fecundity. However, by using radioactively labelled amino acids, Rivero and Casas (1999) showed that a significant proportion of the resources gained by the aphelinid *Aphytis melinus*, a parasitoid of scale insects, were stored and used gradually throughout the life of the wasp. Such techniques are particularly under-utilised in studies of parasitoid behaviour (Sect. 2.13).

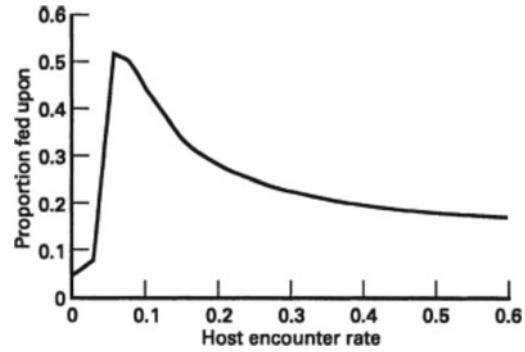


Fig. 1.15 Host feeding by parasitoids. Most models predict that the relationship between the fraction of hosts fed upon and host availability should be dome-shaped, increasing at low levels of host availability and decreasing at moderate to high levels. [The monotonic decline over the mid to high range is supported empirically, e.g., Sahragard et al. (1991).] The functional explanation for the small fraction of hosts fed upon at low host encounter rates is that the female adopts a 'cutting of losses' tactic—the encounter rate is too low to meet (via host feeding) the wasp's energy requirements, and so the female oviposits in every host encountered (Jervis & Kidd, 1986). Models also predict that host feeding is more likely when nutrient reserves and/or gut contents are at or below a critical level. Low nutrient levels and low gut contents presumably warn of the impending risk of starvation and/or egg limitation. In general, the critical level of nutrient reserves/gut contents depends on the current egg load and vice versa (Heimpel & Collier, 1996)

A general prediction of models of destructive host-feeding behaviour is that the fraction of hosts fed upon by female parasitoids should increase with decreasing host availability, at least over the upper range of host densities (Fig. 1.15; Jervis & Kidd, 1986; Chan & Godfray, 1993). This is a prediction borne out by empirical studies (DeBach, 1943; Bartlett, 1964; Collins et al., 1981; Bai & Mackauer, 1990; Sahragard et al., 1991; Thu & Ueno, 2002).

Given that the fitness gains from ovipositing may vary in relation to host stage (Sect. 1.6.7), it is likely that the decision either to host feed or to

oviposit also depends on host stage (Kidd & Jervis, 1991; Rosenheim & Rosen, 1992). Indeed, observational and experimental studies of destructively host-feeding parasitoids show a tendency to feed preferentially or exclusively on earlier host stages and to oviposit preferentially or exclusively on/in later ones (Kidd & Jervis, 1991; Rosenheim & Rosen, 1992; Yang et al., 2012). A similar relationship is likely to apply to different-sized hosts of the same developmental stage.

Furthermore, environmental factors such as temperature may influence rates of host feeding (Urbaneja et al., 2001; Zhang et al., 2019). For example, the egg parasitoid *Trichogramma turkestanica* host feeds on *Ephestia kuehniella* at a greater rate when reared at lower temperatures, although it is not clear why this is so (Hansen & Jensen, 2002). Zhang et al. (2019) found that the temperatures that parasitoids experienced as immatues and as adults affected their host-feeding rates as adults, with the overall amount of host feeding by individuals being highest at intermediate temperatures, but with instantaneous rates being higher at higher temperature as the parasitoids became more active.

Models predict that the decision to host feed *versus* oviposit depends on the parasitoid's egg load: host feeding is more likely when egg load is low (Chan & Godfray, 1993; McGregor, 1997; see Heimpel & Collier, 1996, and Jervis & Kidd, 1999, for reviews). Rosenheim and Rosen (1992) tested this prediction experimentally using the scale insect parasitoid *Aphytis lingnanensis*. Egg load was manipulated by using wasps of different sizes (egg load being a function of body size) and also by holding parasitoids, prior to their exposure to hosts, at different temperatures (the rate of oöcyte maturation and therefore the rate of accumulation of mature eggs in the ovaries being a function of temperature, Sects. 2.3.4 and 2.7.4). Manipulating egg load in this way ensured that previous history of host contact could be eliminated as a possible confounding variable. Alternative methods of manipulating egg load, e.g., depriving parasitoids of hosts or allowing them to oviposit, do not separate the effects of egg load and experience. Rosenheim and Rosen (1992) found in their experiments that egg load did not significantly affect the decision to host feed or oviposit on (small) hosts. However, although egg load was not directly manipulated, more recent work does support the hypothesis that the likelihood of host feeding is related to egg load (e.g., Heimpel & Rosenheim, 1995, Heimpel et al. 1996, Ueno, 1999b). The decision whether to host feed or oviposit may also depend on the wasp's nutritional state (Heimpel & Collier, 1996).

1.9 Host Discrimination

1.9.1 Introduction

Salt (1932) was the first researcher to clearly demonstrate the ability of a parasitoid to discriminate between hosts that contain the egg of a conspecific and hosts that have not been parasitised, and later (Salt, 1961) he showed that this ability, known as host discrimination, occurs in the major families of parasitoid Hymenoptera. Females of some parasitoid species are able to discriminate between: (1) parasitised hosts and unparasitised hosts (numerous published studies have shown this, although the conclusions drawn in some are questionable, see below); (2) parasitised hosts containing different numbers of eggs (Bakker et al., 1990); or (3) hosts containing an egg of a conspecific from one containing their own egg.

Notwithstanding such sophisticated abilities, superparasitism, the laying of an egg in an already parasitised host (Sect. 1.9.4), is a common phenomenon among insect parasitoids. The occurrence of superparasitism or, expressed statistically, the occurrence of a random egg distribution among hosts, has often led to the erroneous conclusion that a parasitoid is unable to discriminate between parasitised and unparasitised hosts (Hemerik & van der Hoeven, 2003, and see below). Dipteran parasitoids rarely show host discrimination abilities, primarily as the females of many species never come into contact

with potential hosts, instead often relying on host-seeking larvae (reviewed in Feener & Brown, 1997; but see Lopez et al., 1995). The effects of superparasitism on progeny development and survival are discussed in Chap. 2 (Sects. 2.9.2 and 2.10.2).

1.9.2 Indirect Methods

There are two approaches to determining whether parasitoids are able to discriminate between parasitised and unparasitised hosts. One is to dissect hosts (Sect. 2.6) and calculate whether the recorded egg distribution deviates significantly from a Poisson (i.e., random) distribution (Salt, 1961). Van Lenteren et al. (1978) have shown that such a procedure is not without pitfalls. They point out that, if the method is applied to egg distributions from hosts collected in the field, there is a risk that mixtures of samples with regular (i.e., non-random) egg distributions but different means may add up to produce a random distribution (Fig. 1.16). This is one of the reasons why a random egg distribution does not constitute proof of the inability to discriminate. Another problem van Lenteren et al. (1978) identified concerning the analysis of egg distributions is that with gregarious parasitoids the distribution of eggs depends not only upon the number of ovipositions but also on the number of eggs laid per oviposition.

There are further problems associated with the use of egg distributions. Van Alphen and Nell (1982) recorded random egg distributions when single females of *Asobara tabida* were placed with 32 hosts for 24 h. Because not all replicates produced random distributions and because other experiments had unequivocally shown that females of this species are able to discriminate between parasitised and unparasitised hosts, the random egg distributions could not be explained by a lack of discriminative ability.

In van Alphen and Nell's (1982) experiments the replicates with a high mean number of eggs had random distributions, whereas replicates with lower means had regular ones. It was therefore concluded that *A. tabida* discriminates between unparasitised and parasitised hosts but is unable to assess whether one or more eggs are present in a parasitised host. Egg distributions are a mixture of the regularly distributed first eggs laid in hosts and of the randomly distributed supernumerary eggs. At lower means, the contribution of the regular distribution of the first eggs is not masked by the random distribution of the supernumerary eggs, whereas at higher means it is.

Even when egg distributions more regular than a Poisson distribution are found, one cannot establish with certainty that a parasitoid is able to discriminate between parasitised and unparasitised hosts. The recorded egg distribution could result from parasitised hosts having a much lower probability of being encountered, either because they move less than healthy hosts or because they leave the host plant. It is also possible that encounter rates with parasitised hosts are lower because the parasitoid does not re-visit previously searched areas with the same probability, e.g., when it always walks upwards along branches or when it marks areas already visited and avoids re-searching such areas.

The previous examples show that there are major pitfalls associated with using egg distributions to determine whether a parasitoid can discriminate between parasitised and unparasitised hosts. Other components of the behaviour of the parasitoid, or of the behaviour of the hosts, can influence egg distributions. Moreover, a regular egg distribution with a mean number of eggs much greater than one requires more than just an ability to discriminate between parasitised and unparasitised hosts. This has already been illustrated in the above-mentioned example of *A. tabida* where no regular egg distributions are found. The following example illustrates how, in *Leptopilina heterotoma*, different mechanisms are responsible for egg distributions tending to be regular even at a high mean number of eggs per host (Bakker et al., 1972). One explanation for this phenomenon is that *L. heterotoma* is able to discriminate between hosts containing different numbers of eggs. There is, however, a second possible interpretation: when the parasitoid is able to distinguish hosts containing an egg of her

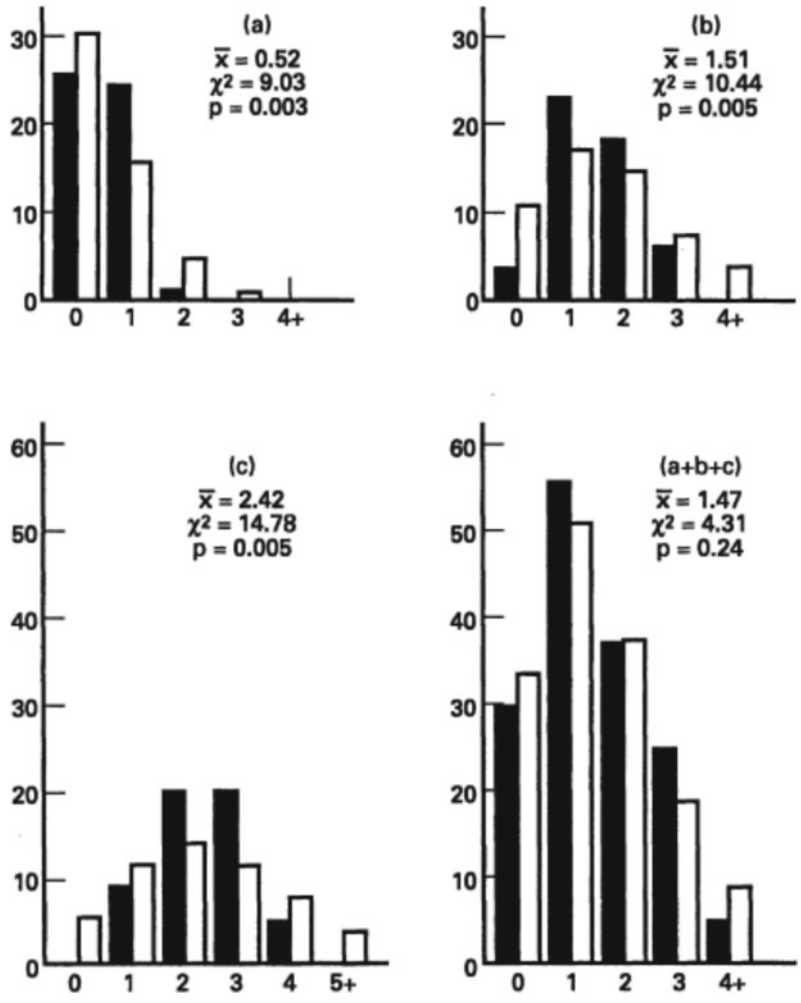


Fig. 1.16 Host discrimination by parasitoids: egg distributions for *Leptopilina heterotoma* (= *Pseudeucoila bochei*) parasitising *Drosophila melanogaster*. Three groups (a, b, c) of around 50 host larvae were presented to female wasps, and the hosts subsequently dissected and examined for wasp eggs. The mean number of eggs recovered per host larva ($\bar{x}$) was different in each case. Although in all three cases (a, b, c) superparasitism occurred, when the egg distribution (black bars) was compared (using χ^2 tests) with the distribution that would have been obtained had the wasps been ovipositing at random (i.e., a Poisson distribution, white bars), the egg distribution was found to be more regular (underdispersed), indicating that the parasitoids discriminate. However, if data from all three distributions are pooled (a + b + c), a distribution is obtained that is indistinguishable from a Poisson distribution (lower right panel), a result that would lead to the erroneous conclusion that the parasitoid species studied cannot discriminate. *Source* van Lenteren et al. (1978), reproduced by permission of Blackwell Publishing

own from those containing eggs of conspecifics and avoids ovipositing in the former, regular egg distributions would result. Therefore, it is not possible to decide, based on egg distributions alone, whether a parasitoid is able to assess the number of eggs already present in a host.

Experiments therefore need to offer a parasitoid female a choice of hosts containing different numbers of eggs, all laid by other (conspecific) females. Bakker et al. (1990) offered hosts containing two eggs and hosts containing one egg of other females to individual

L. heterotoma. The wasps oviposited significantly more often in hosts containing a single egg, thus showing that *L. heterotoma* is indeed able to distinguish between hosts containing different egg numbers. Visser (1992) showed that *L. heterotoma* females are also able to recognise hosts containing their own eggs. Thus, both of the above mechanisms may have contributed to the regular egg distributions found by Bakker et al. (1972).

It is thus clear that, by comparing observed egg distributions with those predicted by a Poisson distribution, one can neither conclude that a parasitoid is able to discriminate between parasitised and unparasitised hosts, nor conclude that it lacks this ability. This does not mean a statistical analysis of egg distributions is useless; it is possible to construct models predicting distributions of eggs for parasitoids having different abilities to avoid superparasitism (e.g., discriminating between healthy hosts and parasitised hosts and counting, discriminating but not counting, discriminating between hosts parasitised by self and hosts parasitised by others), and to compare the theoretical egg distributions with distributions recorded in experiments. Bakker et al. (1972) and Meelis (1982) adopted this approach when investigating whether wasps are able to assess the number of eggs already laid in a host. These authors assumed that parasitoids search randomly, and that there exists a certain probability that the wasp will lay an egg when it encounters a larva. This probability is 1.0 at the first encounter but is lower at subsequent encounters. By keeping the probability of oviposition at the subsequent encounters constant, the model could be used to describe superparasitism by *A. tabida*.

1.9.3 Direct Observations of Behaviour

The other method of determining whether parasitoids are able to discriminate between parasitised and unparasitised hosts involves observing the insects, and recording and comparing encounters resulting in oviposition and rejection of the different host categories. This method provides behavioural evidence that the parasitoid under study rejects parasitised hosts more often than unparasitised hosts. It is, however, wise to use other behavioural criteria in addition to acceptance/encounter ratios.

Because distributions of parasitoid eggs among hosts potentially have an important effect on parasitoid–host population dynamics, one requires a good statistical description of those distributions, for incorporation into population models. We prefer to use the observed behaviour as a basis for a model calculating egg distributions, instead of inferring the underlying behaviour from an analysis of the egg distributions.

More than three decades after host discrimination by hymenopteran parasitoids was discovered, evidence was found of host discrimination by dipteran parasitoids. The phenomenon was described for tachinid flies by Lopez et al. (1995). In field and laboratory experiments, these authors showed that *Myiopharus doryphorae* and *M. aberrans*, both parasitoids of Colorado beetle (*Leptinotarsa decemlineata*) larvae, almost always reject parasitised larvae, whereas they readily oviposit in unparasitised larvae. While little is known of the host discrimination ability of non-hymenopteran parasitoids, even less is understood of the situation where it is the parasitoid larva, rather than the ovipositing female, that actively seeks hosts. Larvae of the staphylinid parasitoid *Aleochara bilineata* locate and attack fly pupae. Royer et al. (1999) found that these larvae can distinguish hosts that were self-parasitised from those that were attacked by conspecifics, and that this was based on chemical cues. Superparasitism was more common when hosts were scarce, and if given a choice, *A. bilineata* larvae would preferentially attack hosts that contained the related species *A. bipustulata*, rather than conspecifics (Royer et al., 1999).

Edwards and Hopper (1999) took a novel approach to investigate levels of superparasitism by *Macrocentrus cingulum*, a braconid parasitoid of the European corn borer, *Ostrinia nubilalis*. Since *M. cingulum* is polyembryonic, the number of parasitoid larvae present per host does not reflect the number of females that have attacked

that host. By using random amplified polymorphic DNA (RAPD) markers (Chap. 3), the authors were able to identify the number of females that had oviposited.

1.9.4 Superparasitism

Many, if not all, parasitoids are able to discriminate between parasitised and unparasitised hosts, but superparasitism is a common feature in nature (van Alphen & Visser, 1990; Godfray, 1994; D'Auro et al., 2021), posing the question: why and when should parasitoids superparasitise?

Van Lenteren (1976) addressed this question from the standpoint of causation. He assumed that superparasitism was caused by a failure to discriminate. He found that females of *L. heterotoma* inexperienced with unparasitised hosts readily oviposited in already parasitised hosts but avoided ovipositing in parasitised hosts after they had been able to oviposit in unparasitised ones. He concluded from this that parasitoids superparasitise because they are unable to discriminate between parasitised and unparasitised hosts until they have experienced oviposition in unparasitised hosts. A similar conclusion was drawn by Klomp et al. (1980) for *Trichogramma embryophagum*.

A functional approach to the problem is to ask whether it is adaptive for a parasitoid always to avoid superparasitism. Van Alphen et al. (1987) re-analysed the data of van Lenteren (1976) and Klomp et al. (1980), starting with the hypothesis that superparasitism can be adaptive under certain conditions. They reasoned that host discrimination is an ability which the parasitoid can use to decide either to reject a parasitised host or to superparasitise it, depending on the circumstances, i.e., superparasitism is not the result of an inability to discriminate. Van Alphen et al. (1987) argued that an inexperienced female arriving on a patch containing only parasitised hosts should superparasitise, because the probability of finding a better patch elsewhere is low. In a similar vein, Sirot et al. (1997) showed through modelling that the tendency to superparasitise should vary with egg load and life expectancy.

Van Lenteren's (1976) inexperienced wasps rejected hosts previously parasitised by themselves more often than unparasitised ones and encountered significantly fewer hosts in experiments involving patches containing only parasitised hosts compared with similar experiments involving patches containing the same density of unparasitised hosts. It was known that *Leptopilina heterotoma* females search by stabbing with the ovipositor, twice per second, in the substrate, and it was possible to measure both the surface area of a host and that of the patches. It was possible therefore to calculate, from the numbers of encounters observed during a 30-min observation period, that inexperienced wasps spent on average 13.12 min searching and handling hosts when introduced on to a patch with parasitised hosts, whereas they spent on average 2.14 min when introduced on to patches with unparasitised hosts. Van Alphen et al. (1987) interpreted the differences in behaviour between inexperienced wasps and experienced wasps as evidence that inexperienced wasps do recognise parasitised hosts and thus concluded that host discrimination does not need to be learnt. Experiments by van Alphen et al. (1987), involving *L. heterotoma* and *Trichogramma evanescens*, confirmed that females inexperienced with unparasitised hosts are, like experienced wasps, already able to discriminate, although inexperienced females superparasitise more frequently. This example shows that alternative hypotheses can be overlooked if one asks only causal questions.

Static and dynamic optimality models as well as ESS models (Sect. 1.2.2) have shown that superparasitism is often adaptive (Iwasa et al., 1984; Parker & Courtney, 1984; Charnov & Skinner, 1985; Hubbard et al., 1987; van der Hoeven & Hemerik, 1990; Visser et al., 1990; Field & Keller, 1999; Hemerik et al., 2002;). The models predict that oviposition in already parasitised hosts, though resulting in fewer offspring than ovipositions in unparasitised hosts, may still be the better option when either there is

no time available to search for and locate unparasitised hosts or when unparasitised hosts are simply not available. By ovipositing into an already parasitised host under such conditions, a female may increase her fitness if there is a finite chance that her progeny will out-compete the other progeny (Sect. 2.10.2). Experimental tests of some of these models have shown that parasitoids behave in such a way that the models' predictions are at least met qualitatively (Hubbard et al., 1987; Visser et al., 1990; van Alphen et al., 1992). For example, Sirot et al. (1997) tested predictions that superparasitism by *Venturia canescens* would be less common if females were provided with food, reducing their risk of mortality. As predicted, superparasitism rates were correlated with egg load and previous access to (non-host) food.

Attacking previously parasitised hosts is evidently adaptive if females can kill parasitoid eggs or young larvae present in the host. *Encarsia formosa* can kill eggs present in hosts by grabbing them with her ovipositor (Netting & Hunter, 2000). A similar effect is seen with *Haplogonatopus atratus* (Dryinidae), where the female wasp kills parasitoid larvae present in the host before ovipositing (Yamada & Kitashiro, 2002). Similarly, ectoparasitoids may eat eggs already present on a host, or kill feeding larvae by pulling them from the host, before laying their own clutch (e.g., Goubault et al., 2007b).

Female parasitoids can often discriminate between hosts that have been self-parasitised from those that have been attacked by a conspecific. *Venturia canescens* females avoid superparasitising hosts that contain their own progeny, a behaviour mediated by the presence of a marking pheromone (Hubbard et al., 1987). Such ability to discriminate among hosts led to the suggestion that females would increase their inclusive fitness by avoiding hosts that contain kin (Fellowes, 1998), and indeed, female *V. canescens* avoid attacking hosts containing relatives (Marris et al., 1996). However, *V. canescens* is parthenogenetic, and this may be an example of extended self-recognition, rather than kin discrimination. Ueno (1994) studied the behaviour of *Itoplectis narayanae* and found that whereas females would avoid parasitising hosts they had previously attacked, there was no difference in their likelihood of attacking hosts that contained kin or unrelated conspecifics.

While in the examples above the females recognise their own odour marks, others distinguish between self-parasitised hosts and those attacked by conspecifics by different means. Ueno and Tanaka (1996) found that *Pimpla nipponica* females do not deposit chemical markers, but instead use visual location cues to avoid self-superparasitising.

Avoidance of self-superparasitism may be one reason that patches are incompletely exploited (e.g., Outreman et al., 2001). With *Venturia canescens*, the likelihood of avoiding superparasitism increases in the 20 min after oviposition if the females have been provided with alternative hosts during the interval, but this does not occur if the female is deprived of other hosts. This suggests that the females can rapidly obtain information on the number of hosts in the patch, and this influences their decision to superparasitise (Hubbard et al., 1999). *Anaphes victus*, a mymarid parasitoid of curculionid beetle eggs, can learn to avoid marked hosts in 4 h, and are quicker to learn if the mark was made by a close relative (van Baaren & Boivin, 1998).

It is unclear where the oviposition deterrent marker originates, although it is usually suggested that it originates from the female's Dufour's gland. The pteromalid *Dinarmus basalis* avoids superparasitising hosts that have been attacked over 20 h previously. Gauthier and Monge (1999) found that the marker originated from the parasitoid egg and required contact between the egg and the host for at least 4 h before the deterrent effect became evident. However, with *Leptopilina boulardi* and *Asobara tabida*, parasitoids of drosophilids on fermenting substrates, the mark spreads within the host within about a minute (van Alphen & Nell, 1982).

Experience is often important in determining whether a female superparasitises a potential host. Naïve *Cotesia flavipes* females readily attack hosts that contain a conspecific, yet experienced females will reject such hosts. This

discrimination is influenced by the presence of a patch-marking odour (Potting et al., 1997). Nufio and Papaj (2001) review patch-marking behaviour in parasitoids.

When parasitoids attack a host that already contains a developing conspecific, the likelihood is that the larvae within the host will fight to the death for ownership of the resource. While it may be expected that older larvae will have a competitive advantage, this does not appear to be the case with *Venturia canescens*, where first instar larvae are more likely to kill older larvae in the same host (Marris & Casperd, 1996). This result appears to explain why the level of superparasitism by *V. canescens* females is higher the longer the period of time that has elapsed since the host was first attached.

While superparasitism is now typically seen as an adaptive aspect of parasitiod foraging behaviour (e.g., Visser, 1993; D'Auro et al., 2021), it may be greatly influenced by viral infection, with virus transmitted both vertically (mother–daughter) and horizontally (between immature parasitoids developing in the same host) and with infected individuals engaging in superparasitism. Superparasitism thus leads to the horizontal transmission of the virus, and may well increase the fitness of the virus at the expense of that of the wasp (Varaldi et al., 2003; Varaldi & Lepetit, 2018).

1.9.5 Multiparasitism

Multiparasitism (oviposition in a host attacked by heterospecifics) has been less studied than superparasitism. In general, it is thought that the ability to identify hosts attacked by other species is less frequent than discrimination against hosts attacked by conspecifics. There are two main situations where females should discriminate against hosts containing a heterospecific egg or larva. First, competitively inferior species should avoid attacking hosts where a superior competitor has already oviposited (Vyas et al., 2019; Sect. 2.10.2). Second, where the outcome of competition depends upon the time since the host was initially attacked (Sect. 2.10.2), the multiparasitising female should be able to detect this factor and incorporate it when making the decision of whether to parasitise or not.

Ueno (1999c) tested this latter prediction, using *Pimpla nipponica* and *Itoplectis naranyae*, two solitary parasitoids of moth larvae. When presented with *Galleria mellonella* larvae, both species preferred attacking previously unattacked hosts when the time since parasitism of the host by the heterospecific parasitoid was over 48 h. However, if less than 24 h had passed since the initial attack, then no such preference was shown. How the parasitoids can distinguish the time since the initial parasitism is not known. Bokonon-Ganta et al. (1996) found that competitively inferior species do not always avoid ovipositing in hosts previously attacked by a competitor. *Gyranusoidea tebygi*, a parasitoid of the mango mealybug, *Rastrococcus invadens*, readily accepts hosts that have previously been attacked by *Anagyrus mangicola*, although their offspring generally fail to survive. Conversely, competitively superior species may prefer hosts parasitised by an inferior competitor (Aguirre et al., 2021).

1.9.6 Cannibalism

In many ways, cannibalism by predators can be considered analogous to superparasitism. Cannibalism is a common feature of the behaviour of many predatory insects and is probably a consequence of polyphagy (New, 1991; Dostalkova et al., 2002). Consuming unrelated conspecifics will have two main benefits (Polis, 1981; Elgar & Crespi, 1992; Anthony, 2003). First, when resources are scarce, the added nourishment gained will increase the survival chances of the cannibal (e.g., the green lacewing *Chrysoperla carnea*, Duelli, 1981). Second, potential competitors are removed from the patch. When alternative resources are common, then it is unlikely that consuming relatives will be beneficial, but when resources are limiting it may be better to eat kin so that some individuals survive, rather than sacrificing all (Fellowes, 1998).

Cannibalism has been most intensively studied in the Coccinellidae, where some species can

complete their larval development on conspecific eggs (Dimitry, 1974). *Adalia bipunctata* will frequently consume conspecifics (Hodek & Honěk, 1996), although adult females and young larvae will avoid their own and sibling eggs, respectively (Agarwala & Dixon, 1993). Males that fathered the eggs do not show any such discrimination. In many non-social insects, such avoidance would be explained by environmental cues, rather than through direct genetic cues (Fellowes, 1998). Joseph et al. (1999) investigated these cues using the ladybird *Harmonia axyridis*. Third-instar *H. axyridis* larvae avoid cannibalising kin, and when they do cannibalise them, they take longer to attack kin than non-kin. These results suggest that environmental cues are unimportant, with discrimination linked to genetic differences among the individuals (Joseph et al., 1999).

Given that there will be heterogeneity in habitat quality, it is perhaps unsurprising that there is heritable variation in cannibalistic behaviour in *H. axyridis* (Wagner et al., 1999). When conditions are favourable, cannibalism is maladaptive given that foraging larvae are more likely to encounter kin. However, in unfavourable patches, increased propensity to cannibalism will increase the development rate and survivorship of the cannibal (Wagner et al., 1999).

1.9.7 Intraguild Predation

If we consider that cannibalism is analogous to superparasitism, then it is reasonable to compare intraguild predation to multiparasitism. Intraguild predation, IGP, is a combination of predation and competition, and occurs when two predators share a common prey species, but one (or both) of the predators will also attack the other (Polis et al., 1989; Arim & Marquet, 2004), resulting in a 'trophic loop'. The study of IGP thus connects research on forgaging behaviour to research on the structure and function of ecological communities, in particular the properties of trophic webs (Marques et al., 2018; Blue Pahl et al., 2020; Aguirre et al., 2021; Chap. 6). IGP interactions are likely to be common, with many adult predators attacking the eggs and the larval stages of other species, as well as their own (Rosenheim et al., 1995). The effects on the suppression of hosts achived by biological pest control are predicted to be negative or neutral, but there is little empirical evidence for the predicted negative effect (Jansen et al., 2006; Aguirre et al., 2021). Here we briefly review several examples of IGP interactions between predators, between parastioids and also between predators and parasitoids.

The anthocorid bug *Orius laevigatus* is frequently used to control the thrips *Frankliniella occidentalis*, a pest of many greenhouse crops. Phytoseiid mites, such as *Neoseiulus cucumeris*, are also used in thrips control. Wittmann and Leather (1997) found that, due to intraguild predation by *O. laevigatus* on *N. cucumeris*, the use of both predatory agents together was unlikely to increase the degree of control. However, *O. laevigatus* does not prey upon another predatory mite (*Iphiseius degenerans*), making a pairing much more suitable for *F. occidentalis* control (Wittmann & Leather, 1997). Similarly, Tsuchida et al. (2022) found that IGP between two species of predatory mites could lead to reduced control of pest mite populations. In another mite system, Marques et al. (2018) found that the adults of two co-occurring predatory species, *Iphiseiodes zuluagai* and *Euseius concordis*, feed on juveniles of the other species, whether or not their shared feeding resource is present, and that adult *I. zuluagai* also attack adult *E. concordis* individuals. These IGP interactions result in *E. concordis* populations failing to persist unless the environment has a sufficiently complex spatial structure. However, under some conditions it is the populations of *I. zuluagai* that tend not to persist. The fact that the outcome of IGP interactions is conditional on the physical environment illustrates the challenges of assessing their importance in natural and agroecosystems, as experiental observations might not be relevant to field conditions.

IGP between parasitoids typically involves facultative hyperparasitism (the offspring of one parasitoid developing on another parasitoid) or predation (one parasitoid feeding as a predator of the other) but may also be intertwined with

aspects of resource competition (determining which parasitoid feeds on the host). These classes of interactions may be termed 'intrinsic competition' when occurring between parasitoid larvae in the same host and 'extrinsic competition' when occurring between foraging adult females (Cusumano et al., 2022a, 2022b).

The role of competition and IGP between two endoparasitoids, *Anagyrus cachamai* and *A. lapachosus* (Encyrtidae), candidates for biocontrol of the Puerto Rican cactus pest mealybug, *Hypogeococcus* sp. (Pseudococcidae), was studied by Aguirre et al. (2021), using Bayesian model selection for statistical analysis to infer difficult-to-observe parasitoid–parasitoid instinsic competion interactions. They found that the species differed in their ability to compete, and in multiparasitsm decisions (Sect. 1.9.5) and functional responses (Sect. 1.14), indirectly indicating IGP of *A. lapachosus* on *A. cachamai* (most likely by acting as a predator rather than as a hyperparasitoid), and also that a multiple release strategy for both the parasitoids would produce better suppression of *Hypogeococcus* sp. than a single species release. In another studied endoparasitoid–endoparasitoid interaction, *Ooencyrtus telenomicida* (Encyrtidae) and *Trissolcus basalis* (Platygastridae) attacking the pentatomid bug, *Nezara viridula*, differed in ther intrinsic and extrinsic competitive abilities, such that coexistence is promoted and biocontrol may not be disrupted, even though *O. telenomicida* is a facultative hyperparasitoid of *T. basalis* (Cusumano et al., 2013, 2022). However, facultative hyperparasitism by one of several ectoparasitoids attacking the coffee berry borer (*Hypothenemus hampei*), alongside readily observable extrinsic competitive interspecific interactions, is likely to be disruptive to biological contol (Pérez-Lachaud et al., 2004; Batchelor et al., 2005, 2006; Sect. 1.13).

IGP by the anthocorid bug *Cardiastethus exiguus* on the larvae of two parasitoids of *Opisina arenosella*, a pest of coconut, *Bracon brevicornis* (Braconidae) and *Goniozus nephantidis* (Bethylidae) has been reported (Nasser & Abdurahiman, 1990) through competitive interactions between the two parasitoids themselves (Hardy & Blackburn, 1991). When, in laboratory studies, adult *G. nephantidis* females encounter foraging *C. exiguus* in the presence of a host/prey larva, the parasitoid attacks the predator, sometimes killing it, but predators are not aggressive towards parasitoids. Despite parasitoid aggression, suriving predators sometimes manage to consume the parasitoid's eggs that had been laid onto the host. Such IGP interactions may reduce the overall suppression of the pest (Velasco-Hernandez et al., 2021).

1.10 Clutch Size

Since a host represents a limited amount of resource, and parasitoid offspring have the potential to compete for that resource (Sects. 2.9 and 2.10), gregarious parasitoids must make an additional decision after accepting a host for oviposition: how many eggs to lay in (or on) a host. Many studies have addressed this question (e.g., Skinner, 1985; Waage & Godfray, 1985; Waage, 1986; Godfray, 1987a, 1987b; Hardy et al., 1992b; Vet et al., 1994; Visser, 1996a, 1996b; Zaviezo & Mills, 2000; Bell et al., 2005; Goubault et al., 2007a; Hasan & Ansari, 2010; Villacañas de Castro & Thiel, 2017; Samková et al., 2022). Here we are mainly concerned with variation in the size of clutches allocated to hosts of a fixed size, although we shall also consider host size variation.

Given that the amount of resource a developing parasitoid obtains will determine its fitness, a fitness function $f(c)$ can be used to describe the fitness of each offspring in a clutch of size c allocated to hosts of a certain size. The fitness gain to the mother per host attacked is therefore the product of clutch size and the *per capita* fitness function, i.e., $cf(c)$. The value of c where $cf(c)$ is maximised is the parental optimum clutch size, known as the 'Lack clutch size', after Lack (1947) who studied clutch size in birds. Predicted and observed fitness functions for three parasitoid species are shown in Fig. 1.17. In each case, the probability of survival to the adult stage is used as the measure of fitness.

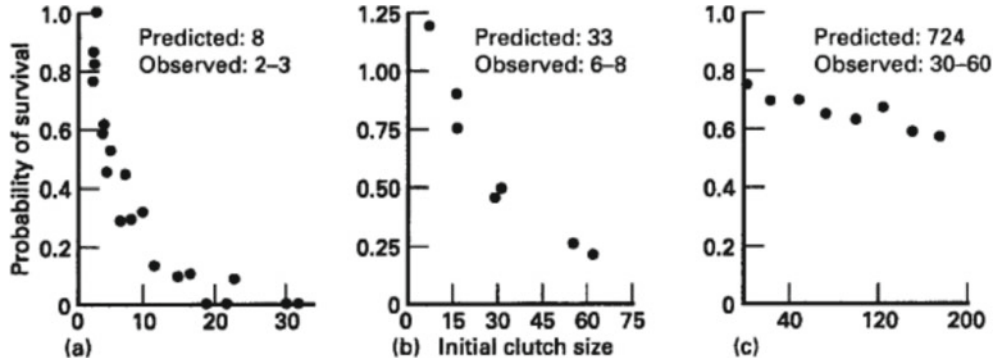


Fig. 1.17 Optimal progeny allocation in gregarious parasitoids—clutch size: *per capita* fitness of offspring as a function of clutch size, estimated by the probability of survival in initial clutches of different sizes (Observed = observed clutch size; Predicted = predicted by calculation of *cf*(*c*); see text). **a** *Trichogramma evanescens* in eggs of the cabbage moth, *Mamestra brassicae*; **b** *Telenomus farai* in eggs of the bug *Triatoma phyllosoma pallidipennis* (the overestimate of survival [>1.0] in this case is attributable to sampling error); **c** *Dahlbominus fuliginosus* on pupae of the sawfly *Neodiprion lecontei*. *Source* Waage and Godfray (1985) and Waage (1986), who used data from Pallewatta (1986), Escalante and Rabinovich (1979) and Wilkes (1963). In all three cases, there is a continuous decline in *per capita* fitness with increasing clutch size. For some other gregarious parasitoid species there is evidence of an Allee effect, i.e., an initial rise then a fall in fitness. Such a dome-shaped fitness relationship may prove to be common among gregarious endoparasitoids, because in such parasitoids small larval broods often perish entirely due to their inability either to overcome host physiological defences or to consume all the host tissues (a prequisite in some species for successful pupation and emergence). Reproduced by permission of Blackwell Publishing and Elsevier Science

Fitness function curves can be constructed as follows:

1. By exposing hosts to individual parasitoids and examining/dissecting some of these hosts immediately after oviposition to determine clutch size, and rearing parasitoids from the remainder to determine offspring survival (Fig. 1.17a, b). If larval mortality arising from resource competition occurs late in development, and dead larvae are not consumed by surviving larvae, one may simply record the numbers of emerged and unemerged offspring (Fig. 1.17c).
2. By manipulating parasitoid clutch sizes. This is relatively easy in the case of ectoparasitoids, as different clutch sizes can be obtained simply by adding or removing eggs, manually, from clutches present on the host's body surface (Hardy et al., 1992b; Zaviezo & Mills, 2000; Milonas, 2005; Villacañas de Castro & Thiel, 2017). With this technique, however, there is a risk of damaging eggs during manipulation. It may be possible to deal with this problem by using the number of larvae that successfully hatch from the manipulated eggs as a proxy for clutch size, rather than the manipulated clutch size itself. With endoparasitoids, clutch sizes can be manipulated by interrupting oviposition, by allowing superparasitism to occur, or by exchanging the host for one of a different size after the wasp has examined it but immediately before it has the opportunity to begin ovipositing in it (Klomp & Teerink, 1962). However, a problem with at least the latter technique is that the parasitoid may alter its sex allocation behaviour and the sex ratio of the clutch of eggs may influence progeny fitness and thus also the optimum clutch size (Waage & Ng, 1984).

Other models predict that the best strategy for a parasitoid is to maximise fitness per unit time rather than per host attacked. If there is a cost in time to laying an egg, it may benefit a female to cease adding more eggs to a host and to allocate the time saved to locating a new host. The fitness gain from leaving hosts and searching for new

ones will increase as the travel time between oviposition sites decreases, i.e., as host availability increases. As hosts become more abundant, females should leave each host sooner, i.e., produce smaller clutch sizes. Thus, with the maximisation of fitness per unit time models, females maximise fitness per host attacked (i.e., produce Lack clutch sizes) only when hosts are scarce (Godfray, 1994). *Trichogramma minutum* appears to be a species whose strategy is to maximise fitness per unit time. Schmidt and Smith (1987a) presented females with nine host eggs attached by glue to a cardboard base and employed various egg spacing treatments: the eggs were situated with their centres either 2, 3, 4 or 5 mm apart on a grid. Clutch size was found to decrease with increased crowding of eggs, i.e., increasing host density per unit area.

There are also models that take into account egg-limitation constraints, i.e., they assume that the parasitoid has a limited number of eggs to lay at any one time. Such a parasitoid is always in a position where available eggs are fewer than potential clutch sites. If eggs are severely limiting, i.e., egg load is much smaller than the number of hosts available (this could be due to the fact that the female has laid most of her eggs, e.g., Heimpel et al., 1998), a female should spread out her eggs between hosts so that the fitness gain per egg, rather than per clutch, is maximised. When *per capita* fitness of offspring decreases monotonically (as in Figure 1.17), the optimal clutch size under severe egg limitation is always one (see Zaviezo & Mills, 2000, for the effects of female life expectancy on optimal clutch size).

What if data and model predictions do not match? As can be seen from Fig. 1.17, clutch sizes predicted by optimality models tend to differ from the ones recorded in experiments. This discrepancy may occur for one or several reasons:

1. The wrong fitness currency has been used. For example, the parasitoid's strategy may be that of maximising fitness per unit time rather than per host attacked, or a classic (static or dynamic) optimality model has been used rather than a game-theoretic model (see below).
2. The model does not take account of stochastic variability in certain parameters (Godfray & Ives, 1988).
3. The measure of fitness (e.g., offspring survival to adulthood) used may be inappropriate or incomplete (e.g., Visser, 1994).
4. The measure of fitness is appropriate but difficult to quantify (e.g., laboratory estimates do not accurately reflect field estimates, e.g., Visser, 1994; West et al., 1996).

If the fitness measure, such as juvenile survival, is inapproproiate, measures such as adult fecundity or longevity, both influencing foraging performance, may be more important. Calculating fitness as total offspring fecundity may lead to a closer fit between model and data (Waage & Ng, 1984; Waage & Godfray, 1985). Measuring offspring fecundity is likely to prove very time-consuming, so an alternative procedure is to measure offspring body size or weight; both of these factors are usually good predictors of fecundity in parasitoids (Sect. 2.7.3). Le Masurier (1991) used a combined measure of fitness: the product of progeny survival and the calculated mean egg load at emergence (a measure of lifetime fecundity) of the surviving female progeny. The egg load for each emerging wasp was determined indirectly, from a regression equation relating egg load to head width. Le Masurier (1991) found that the fitness function curve constructed for a British population of *Cotesia glomerata* in larvae of *Pieris brassicae* showed no density-dependent effect of clutch size on fitness, and this therefore prevented him from calculating the optimum clutch size for that host: all that could be predicted was that females should lay at least the maximum number of eggs recorded in a host.

Release-recapture experiments with different size classes of parasitoids in the field may provide useful information on size–fitness relationships, although ideally these relationships should be directly measured (Visser, 1994; West et al., 1996; Ellers et al., 1998).

It has become increasingly apparent that measurements of size–fitness relationships are strongly influenced by environmental variation (Rivero & West, 2002). In addition, laboratory studies tend to underestimate the disadvantages of small body size in parasitoid wasps (Hardy et al., 1992b; Visser, 1994; West et al., 1996). Therefore, any assumption that there is a general size–fitness correlation in parasitoids needs to be treated with caution. Furthermore, the fitness of an individual with a given body size may be dependent on the sizes of other individuals in the population. This occurs, for instance, when foraging females compete directly, via agonistic interactions, for access to hosts (Sect. 1.13) and the probability of gaining or retaining access is higher for relatively large competitors. If such competition occurs frequently, mothers are expected to produce smaller clutches that generate fewer but larger offspring, in response to the sizes of clutches (and offspring) being produced on other hosts by other mothers. The optimal clutch size in these circumstances is an ESS (Sect. 1.2.2), found by game theory, rather than a prediction of the classical optimality approach (Petersen & Hardy, 1996; Mesterton-Gibbons & Hardy, 2004; Goubault et al., 2007a).

A further factor to consider is variation in host size. If a gregarious parasitoid's strategy is that of maximising fitness per host attacked, then the optimal clutch size ought to increase with increasing host size. Gregarious parasitoids do tend to lay larger clutches in or on larger hosts, both within and across species (e.g., Hardy et al., 1992b; Mayhew & Hardy, 1998; Shameer et al., 2002; Wang et al., 2008; Kapranas et al., 2011; Malesios & Prophetou-Athanasiadou, 2014; Tang et al., 2014; Villacañas de Castro & Thiel, 2017). How do gregarious (and solitary) parasitoids measure host size? Schmidt and Smith (1985) studied host size measurement in *Trichogramma minutum*. Females allocated fewer progeny to host eggs that were partially embedded in the substratum than into host eggs that were fully exposed. Since the eggs were of identical diameter and surface chemistry, it was concluded that the mechanism of host size determination is neither chemosensory nor visual, but is essentially mechanosensory, based on accessible surface area. Schmidt and Smith (1987b) subsequently observed the behaviour of individual *T. minutum*, during the host examination phase, on spherical host eggs of a set size, and recorded: (1) the frequency of and intervals between contacts with the substratum bearing the eggs, and turns made by the wasps, and (2) the number of eggs laid per host. In analysing the data, seven variables were considered: the total number of substratum contacts, the mean interval between such contacts, the interval between the last contact and oviposition, the longest and shortest interval between contacts, the total interval between the first three contacts, and the interval between the first contact with the host and the first contact with the substratum (initial transit). Of these, only the duration of the initial transit across the host surface showed a significant positive linear relationship with the number of eggs deposited. By interrupting the path of wasps during their initial transit, and thereby reducing their initial transit time, Schmidt and Smith (1987b) succeeded in reducing the number of progeny laid by a female. Schmidt and Smith (1987b) concluded that wasps are able to alter progeny allocation by measuring short time intervals. Interestingly, the duration of initial transit was found to be the same for both large and small wasps (Schmidt & Smith, 1987b, 1989).

Large-bodied gregarious parasitoids (and solitary parasitoids) are likely to measure host size in other ways, for example by determining whether the tips of the antennae reach certain points on the host's body. Such stimuli are thought to be tactile (e.g., King, 1998). Alternately, simple visual examination of the whole host may provide the correct cues.

Finally, using the isofemale line method, Wajnberg et al. (1989) demonstrated a significant intra-population genetic variation in the clutch size laid by *Trichogramma maidis* (= *T. brassicae*) females and the distribution of eggs within *Ephestia kuehniella* host eggs. This demonstrates that the trait can be the target of natural selection, leading to optimal clutch sizes in different environmental situations.

In this section we have focused on clutch size as an optimality problem for foraging natural enemies. We note that observed clutch sizes of parasitoids and predatory insects may be influenced by a wider suite of constraints and considerations than are covered here, such as phylogeny, anatomy and even geometry (e.g., Mayhew & Hardy, 1998; Abram et al., 2023).

1.11 Sex Allocation

1.11.1 Introduction

Haplodiploidy (the production of haploid males from unfertilised eggs and diploid females from fertilised eggs; also known as arrhenotokous parthenogenesis, Sect. 3.3.2) allows female wasps to determine the sex of their offspring. Such control of sex allocation has made the parasitoid Hymenoptera a favoured subject for behavioural ecologists studying adaptive sex allocation (Godfray, 1994; Godfray & Shimada, 1999; Ode & Hunter, 2002; Ode & Hardy, 2008; West, 2009; Hardy & Boulton, 2019; Abe et al., 2021). There is much more to this topic than can be covered in any detail here: Chaps. 3, 4 and 5 also contain discussions of sex allocation and closely associated topics.

Natural selection will normally favour equal investment in the sexes in an outbreeding (panmictic) population (Fisher, 1930; Gardner, 2023) (Fig. 1.18). Many hymenopterans, however, frequently exhibit sex ratios that are strongly divergent from equality: explaining these differences has resulted in a robust and intricate set of models that are well, and reciprocally, supported by empirical investigations (Godfray, 1994; West, 2009; Gardner & Hardy, 2020; Abe et al., 2021; Lehtonen et al., 2023). In general, patterns of sex allocation are influenced by two main factors: the population's mating structure and the environmental conditions experienced (Hardy & Boulton, 2019). While both can select for the ability to maximise fitness through manipulation of offspring sex ratio, the patterns of sex allocation they influence are quite different.

Fig. 1.18 Sex allocation theory for panmictic and for locally mating species. The optimal primary sex ratio (proportion of offspring that are males) in relation to the number of foundress females (mothers) exploiting a patch. The dashed line represents the 'Fisherian' scenario of population-wide mating among maturing offspring (panmixis) leading to selection for equal investment in sons and daughters by mothers: even sex ratios maintained by frequency-dependent selection (Fisher, 1930; Gardner, 2023). The solid line represents the 'Hamiltonian' scenario of strict local mating (LMC) by offspring within their natal patch followed by the dispersal of mated daughers only (Hamilton, 1967): the optimal sex ratio for individual mothers (termed foundresses) depends on sex allocation decisions by other mothers on the patch and the unbeatable solution for them all to adopt (the ESS) is given by $(n - 1)/2n$, where n is the number of females colonising the patch. For the single-mother case, the prediction of a sex ratio of zero is taken to mean 'just sufficient males to mate with all daughters in the patch'. The LMC prediction shown is for diplo-diploid genetics; the prediction for haplo-diploid genetics is that sex ratios are slightly more biased. The models summarised here form much of the core of sex allocation theory (consisting of many empirically justified modifications), which has become a very successful area within evolutionary biology

1.11.2 Local Mate Competition

The first broad pattern that needs to be explained is a cross-species one: sex ratios (usually expressed as the proportion of the progeny that are male) can vary from highly female biased to equality, or much more rarely, become male-biased. For example, Bernal et al. (1998) reported that mated female *Coccophagus semicircularis* produce strongly female-biased sex ratios; the *Drosophila* parasitoid *Leptopilina*

heterotoma has a sex ratio near equality, whereas the closely related species *L. boulardi* has a male-biased sex ratio (Fauvergue et al., 1999).

This variation has been explained by the theory of local mate competition (Hamilton, 1967; Godray, 1994; West, 2009). Under conditions of Local Mate Competition (LMC) ovipositing females are predicted to lay an increasingly female-biased offspring sex ratio as the likelihood of sib-mating increases. Imagine a patch where only one female oviposits and lays a given number of eggs and then, on maturity, her sons mate with her daughters, before the sons die and the daughters disperse to find new patches to lay their eggs on. With an unbiased sex ratio, the males in the maturing brood will compete with each other for matings with their sisters. The ovipositing female can increase her fitness by changing the proportions of male and female offspring in her brood, increasing allocation to females to the point where there are only enough males present to ensure that all females in the brood are mated. Biasing the sex ratio in this manner means that competition between sons for access to mates will be lower, the number of available mates for each male will be increased, and the overall production of mated daughters from the brood will be maximised (Antolin, 1993; Ode et al., 1998; West, 2009).

Conditions suitable for LMC are most likely to be met when patches are discrete (the classic example is that of the pollinating fig wasps where only one female will oviposit within each fig fruit; Hamilton, 1979; see Greeff & Kjellberg, 2022, for a review of pollinator wasp sex ratios), when patches are defended by females from attack by other searching females (Sect. 1.13), or when the density of females is low and offspring tend to mate near the emergence site (Hardy, 1994; Chap. 5). With increased numbers of females ovipositing in a patch, the males of different mothers are able to mate with a focal mother's daughter and the focal mother's sons can also mate with the daughters of other mothers. Sex allocation decisions by individual mothers within groups can be seen as a game-theoretic problem, and the ESS (Sect. 1.2.2) is that each female should increase the proportion of males in her brood compared to what she would do if reproducing alone (Hamilton, 1967). When large numbers of females produce offspring on a patch, the optimal sex ratio becomes essentially the same as under population-wide mating systems (panmixis): an equal allocation to male and female offspring. Hamilton (1967) showed that the predicted ESS (which he termed the 'unbeatable' sex ratio) is $(n - 1)/2n$, where n is the number of females colonising a patch of resource, on which their offspring mate at random (Fig. 1.18). This applies to organisms with diplo-diploid genetics; for haplidiploids the equation becomes $(n - 1)(2n - 1)/n(4n - 1)$, giving a very slightly more female-biased sex ratio than the diploid model when multiple mothers colonise a patch (Hamilton, 1979; Taylor & Bulmer, 1980; West, 2009).

In a comparative study of the sex ratios of non-pollinating fig wasps (which are primarily inquilines or parasitoids), Fellowes et al. (1999) used the wing morphology of males to distinguish between species that naturally vary in the levels of LMC experienced. Species with wingless males have to mate within the fig fruit, and hence are likely to experience high levels of local, often sibling, mating. In contrast, species with winged males will mate outside the fig, often after dispersal, resulting in near-random mating thoughout the population. In some species of non-pollinating fig wasps an intermediate proportion of males are winged; these species should experience an intermediate level of local mating (termed partial LMC, Hardy, 1994). Theory predicts that sex ratios should follow the rank order of winged males > dimorphic males > wingless males, and a phylogenetically controlled analysis (Sect. 1.2.3) confirmed this pattern. Further comparative studies of sex allocation under LMC are provided by Griffiths and Godfray (1988), Hardy and Mayhew (1998) and Smart and Mayhew (2009); see aso Mayhew and Pen (2002).

Within species, similar patterns are also found. Indeed, Salt (1936) noted that the proportion of male *Trichogramma evanescens* offspring emerging from hosts increased when more females oviposited in a patch. This observation

was confirmed and developed further by Waage and Lane (1984), who found that while sex ratio did increase with the number of ovipositing females, the sex ratio was more male-biased than expected. They explained this by proposing that females were less likely to survive superparasitism. More recent examples of parasitioid sex ratio responses to the numbers of females reproducing on a patch can be found in, for example, Burton-Chellew et al. (2008), Ode and Hardy (2008), West (2009), Abdi et al. (2020a) and Abe et al. (2021).

1.11.3 Conditional Sex Allocation

The second pattern of sex allocation behaviour that needs to be explained is the preferential oviposition of one-offspring sex (usually female) in better-quality hosts. This was explained by the theory of Conditional Sex Allocation (Charnov, 1979; Charnov et al., 1981; King, 1993; Godfray, 1994; Hardy & Boulton, 2019; West, 2009). The size of parasitoids (especially idiobionts) is often determined by host size. As there is often a positive correlation between parasitoid body size and fitness (Sect. 1.10), larger hosts should be preferred. However, if the size–fitness relationship is stronger for female parasitoids than for males, an ovipositing female will maximise her fitness by placing female eggs in better-quality hosts and males in poor-quality hosts (e.g., King & King, 1994; King & Lee, 1994; Morris & Fellowes, 2002; Ode & Hardy, 2008; Hardy & Boulton, 2019). The relative fitness benefits of larger male and female size are, however, challenging to assess, especially in the field (Karsai et al., 2006; King & Napoleon, 2006; Sect. 2.7.3).

Aphelinus abdominalis is a common parasitoid of several aphid species. In a detailed study, Honěk et al. (1998) investigated the sex allocation behaviour of this parasitoid when presented with several potential host species. In all four aphid species (*Macrosiphum euphorbiae*, *Metapolophium dirhodum*, *Sitobion avenae* and *Rhopalosiphum padi*), females preferentially placed male offspring in smaller hosts. If females were provided with small hosts only, then over time the sex ratio became less male-biased. Interestingly, virgin females (i.e., those constrained to produce male progeny only) initially favoured small hosts, but over time preferentially attacked larger hosts when provided with a choice. Further examples of parasitoid sex allocation responses to host size are reviewed in, for example, Godfray (1994), Ode and Hardy (2008) and West (2009).

1.11.4 Sex Allocation and Mass Rearing

Female-biased sex ratios are clearly the preferable outcome of parasitoid mass rearing for biocontrol programmes, as only female parasitoids attack hosts in the field. In a survey of the sex ratios of parasitoids and predators mass reared for biological control purposes, Heimpel and Lundgren (2000; see also Lundgren & Heimpel, 2003) found that while predators all had an unbiased sex ratio, a large proportion of the parasitoids had a more male-biased sex ratio than expected. Such work suggests that the producers of biocontrol agents may be able to improve the quality of their product with changed rearing techniques. Thus, the study of sex allocation in insect parasitoids is an important area of study not only for those interested in evolutionary ecology but also for practitioners of biological control (Hardy & Ode, 2007; Luck, 1990; Ode & Hardy, 2008), and such considerations have underpinned several studies of parasitoid sex allocation behaviour.

Sagarra and Vincent (1999) suggested that *Anagyrus kamali*, a parasitoid of the hibiscus mealybug, should be reared on larger hosts to maximise the production of female progeny. However, one point that can be missed in such studies is that conditional sex allocation is usually a relative behaviour. Female offspring will be placed in larger hosts, but if only large hosts are presented then the sex ratio will approach equality. Thus, a mother may lay a son in a medium-sized host that is encountered among a batch of large hosts but would lay a daughter in a medium-sized host found among small-sized

hosts. Females are expected to update their estimations of the distributions of host sizes as they encounter a succession of hosts during their lives. Several studies, on *Catolaccus grandis* (Hymenoptera: Pteromalidae) a parasitoid of boll weevils, and *Diglyphus isaea* (Hymenoptera: Eulophidae), which attacks agromyzid leafminers, have shown that increasing the size of hosts presented to females over several days leads to a greater production of female offspring than does presenting similar host sizes each day, and presenting smaller and smaller hosts leads to male-biased sex ratios. The technique works not just with females held in isolation (which would be labour intensive in a mass-rearing facility) but also when hosts are presented to groups of parasitoids. Under simulated mass-rearing conditions the production costs of females can be cut by as much as a half (Ode & Heinz, 2002; Chow & Heinz, 2005, 2006; Ode & Hardy, 2008).

Similarly, attempts have been made to use LMC theory to improve mass-rearing efficiency. Since a major prediction is that sex ratios will be more female biased when fewer females contribute offspring to a patch, rearing programmes can be set up such that females encounter hosts in isolation rather than in the presence of other females. Experiments by Irvin and Hoddle (2006) on three species of Mymarids, parasitoids in the genus *Gonatocerus*, indicated that mass-rearing efficiency can be substantially improved by minimising contact between females presented with hosts.

1.11.5 Density-Dependent Shifts in Sex Ratio

In a population of wasps adjusting sex allocation as predicted by Hamilton's (1967) model, the proportion of male offspring produced per female will be higher at high wasp densities than at low densities. Thus, individual optimisation does not go hand in hand with maximal female production in a population. This is one reason why the mass rearing of parasitoids often does not result in desired female-biased sex ratios (Sect. 1.11.4).

Models like Hamilton's (1967), which predict adaptive shifts in sex allocation in response to the presence of other females, raise the question of how these shifts can be achieved. Waage (1982) was the first to show that simple fixed mechanisms, such as always laying one or more male eggs first (Fig. 1.19), can lead to variable sex ratios under different conditions, close to those predicted by the functional models (Waage & Lane, 1984; Waage & Ng, 1984). Rather than counting the number of hosts in a patch and calculating what fraction of her offspring should be sons, the female can lay a son, then lay the number of daughters he can fertilise, then lay another son, and so on (reviewed in Hardy, 1992, see also Greeff & Kjellberg, 2022). This has been theoretically demonstrated by Wajnberg (1994). Other mechanisms are also known where the stimulus to change the sequence of sex allocation comes from contacts with marks of conspecifics or with parasitised hosts (Viktorov, 1968; Viktorov & Kochetova, 1971).

To show that females adjust sex ratio in response to the presence of other females, offer patches containing equal numbers of standardised hosts to different densities of parasitoid females (as in a mutual interference experiment, Sect. 1.15.3). Primary sex ratio can be determined as described below (Sect. 1.11.6). If females use a simple 'males-first' rule, a shift in sex ratio can be found with this setup. Alternatively, one could offer a fixed number of standardised hosts per female in experiments with different numbers of females. In such an experiment, the number of hosts increases with the number of wasps. Where females use a 'males-first' rule, no sex ratio adjustment should be found unless, that is, females react to parasitoid odour or marks.

Strand (1988) adopted a quite different approach in studying density-dependent shifts in sex ratio in *Telenomus heliothidis*. He kept groups of around 200 females together, without hosts, for variable periods of time shortly after emergence and mating, then allowed subsequently isolated females to oviposit in unparasitised hosts (Strand, 1988, also tested for the effects of subsequent isolation by varying the

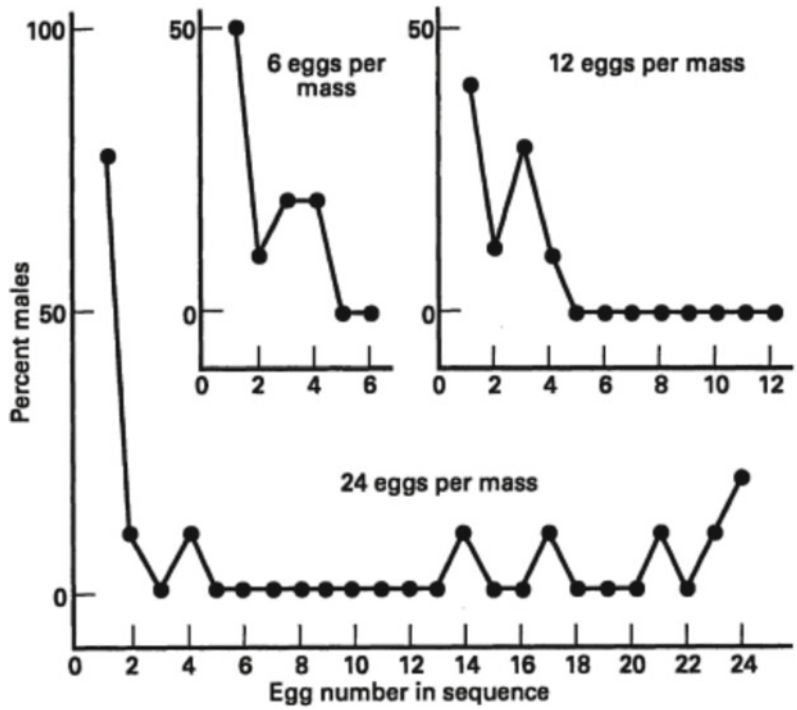


Fig. 1.19 Sex allocation by parasitoids: the sequence in which the solitary scelionid parasitoid *Gryon pennsylvanicum* (= *atriscapus*) lays male and female eggs in host egg masses of 6, 12 and 24 eggs. Data are based on observations of mated females and subsequent dissection of host eggs at the time of adult emergence. In all egg masses, males (usually one per mass) are placed in the first few host eggs. By this strategy, every host egg mass, independent of size, is ensured a male wasp offspring. In very large egg masses, e.g., of 24 eggs, a second male is sometimes produced towards the end of the sequence, suggesting that females measure the ratio of males to females and keep it constant for a particular size of egg mass. The males-first strategy alone will also produce an adaptive increase in sex ratio with increased female crowding, since each wasp will lay fewer eggs per egg mass and therefore allocate proportionately more males. *Source* Waage (1982), reproduced by permission of Blackwell Publishing

isolation period). With this method, one can rule out the possibility that females alter sex ratio in response to encounters with already parasitised hosts. Strand (1988) concluded that the observed effects were directly due to crowding.

Simple mechanisms such as 'males first' can easily be studied in solitary parasitoids by collecting a sequence of hosts parasitised by an individual female and rearing each of the hosts in separate containers. Investigating such behaviour in gregarious parasitoids is more difficult, but by interrupting oviposition at various points during the laying of egg clutches and rearing the parasitoids, information on the sequence of male and female eggs can be obtained. Sometimes the sequence of male and female eggs can be inferred from the behaviour of the female during the oviposition bout (Sect. 1.11.6).

1.11.6 Measuring Primary Sex Ratios

Theories of sex allocation deal with the oviposition decisions of female wasps. Tests of these theories may require accurate measurement of the sex ratio of the oviposited eggs, i.e., the allocated or primary sex ratio. Often, however, due to differential mortality of male and female immatures, the sex ratio of emerging parasitoids, the so-called secondary sex ratio, does not

always reflect the primary sex ratio, and even if mortality is not different between the sexes, mortality can affect the sexual composition of broods and obscure sex allocation strategies (van Baaren et al., 1999; Khidr et al., 2013; Wilkinson et al., 2016; Liu et al., 2023). It is possible, especially when working on ectoparasitoids where egg-to-adult mortality is readily observable, to use a subset of broods in which there was no mortality as an indicator of the primary sex ratio. Although this may serve a useful purpose in some cases (e.g., Werren, 1980), this method is, strictly speaking, flawed because the subset of broods without mortality are a self-selected subset, with sex ratios biased towards the sex with the lowest mortality (Krackow & Neuhäuser, 2008; Khidr et al., 2013, see also Wellings et al., 1986). To take account of this problem, the following empirical methods can be used to obtain unbiased estimates of primary sex ratios:

1. Behavioural indictators: Cole (1981), Suzuki et al. (1984) and Strand (1989) discovered for some species that one can determine, on the basis of differences in the insect's abdominal movements during oviposition, whether or not a female parasitoid fertilises an egg. A feature common to these species is a pause during oviposition of a fertilised (female) egg.
2. Positional indicators: Flanders (1950) and Luck et al. (1982) used a non-destructive method for *Aphytis* that involves determining the sex of an egg from the position in the host in which it is laid: wasps lay male and female eggs on the host's dorsal surface and ventral surface, respectively.
3. Chromosomal counts*:* cytological techniques for counting chromosomes of haplo-diploid parasitoids can be used to establish the sex of freshly laid eggs (Dijkstra, 1986; van Dijken, 1991): further details are given in Sect. 3.4.1.
4. Molecular genetics*:* between-strain polymorphisms in microsatellite markers can be used in crosses between males and females from stain carrying different alleles and then eggs in which only one allele is detected (hemizygous) are identified as male and those containing both alleles (heterozygous diploids) are identified as female (Khidr et al., 2013; Liu et al., 2023; Sect. 3.4.1). De Menten et al. (2003) used fluorescence *in-situ* hybridisation (FISH) to sex ant eggs. This approach has been applied to parasitoid wasps (Carabajal Paladino et al., 2013) but not yet to assess primary sex ratios.

1.12 Switching Behaviour

Species and host stage preference by natural enemies has been discussed in Sect. 1.6.7. Preference (parameter c in Eq. 1.1) may not be constant but may vary with the relative abundance of two prey or host types, in which case if the predator or parasitoid eats or oviposits in disproportionately more of the more abundant type (c increases as N_1/N_2 increases) it is said to display a switching behaviour (Murdoch, 1969) or an apostatic selection (Clarke, 1962), the latter term being used by geneticists. Where disproportionately more of the rarer type is accepted (c increases as N_1/N_2 decreases) negative switching is said to occur (Chesson, 1984). Positive switching behaviour has aroused the interest of students of population dynamics because it is associated with a Type 3 functional response (to prey type N_1) (Sect. 1.14) (Murdoch, 1969; Lawton et al., 1974).

Switching behaviour in parasitoids has been observed by Cornell and Pimentel (1978) in *Nasonia vitripennis*, van Alphen and Vet (1986) in *Asobara tabida*, Chow and Mackauer (1991) in *Aphidius ervi* and *Praon pequodorum*, and probably by Lill (1999), while switching in insect predators has been observed by Lawton et al. (1974) in the waterboatman *Notonecta glauca* and the damselfly *Ischnura*. Other examples are given in Sherratt and Harvey (1993) who provide a comprehensive review of switching and frequency-dependent selection in general.

Switching can be tested for by offering parasitoids combinations of different host species in single-patch experiments. The combined density of the two host species should be kept constant,

but the relative abundance of the two species should vary among treatments. If the mechanism causing the switching is to be determined, full records of parasitoid (and host) behaviour ought to be made. As in other host selection experiments (Sect. 1.6.7) females should be observed continuously and the number of acceptances and ovipositions scored, to show whether females accept more or fewer individuals of a host type than they successfully parasitise (this possibility is usually ignored by authors).

One can either conduct fixed-time experiments, in which depletion of the hosts is prevented by replacing each parasitised host by an unparasitised one of the same species, or terminate experiments when the parasitoid leaves the patch that it is allowed to deplete. Switching, like many other aspects of parasitoid and predator behaviour, is likely to be affected by previous experience of the natural enemy (see below).

The resulting data can be analysed using Murdoch's (1969) null or no-switch model:

$$P_1 = cF_1/(1 - F_1 + [cF_1]) \qquad (1.2)$$

where F_1 is the proportion of host species 1 in the environment, P_1 is the proportion of species 1 among all the hosts oviposited in, and c (a parameter we have already mentioned in Sect. 1.6.7) corresponds to the one used in Eq. (1.1) (Sect. 1.6.7). In the absence of switching behaviour, c is a constant that can be estimated in various ways, although it is convenient to estimate it when $N_1 = N_2$. The value of P_1 for any level of availability of species 1 can be estimated by substituting the estimated value of c in Eq. (1.2), and an expected no-switch curve plotted (Fig. 1.20). If the parasitoid or predator species' preference is not constant but alters with changing host or prey availability (or encounter rate) the observed proportion of host/prey species 1 among all the accepted hosts or prey will be higher than expected when species 1 is abundant and lower than expected when species 1 is rare.

Elton and Greenwood (1970, 1987) and Greenwood and Elton (1979; see also Sherratt & Harvey, 1993) provide a model which can be used for the detection of switching and other forms of frequency-dependent selection, and which includes a measure of the deviation from constant preference as one of the parameters. Another model developed by Manly et al. (1972), and Manly (1972, 1973, 1974; see Sherratt & Harvey, 1993, for a discussion) takes account of prey depletion (this model requires modification before it can be used to take account of depletion of unattacked hosts or prey). The latter model can also be easily generalised for more than one host/prey or host type.

Tinbergen (1960) suggested, as a mechanism for switching, that predators form a search image

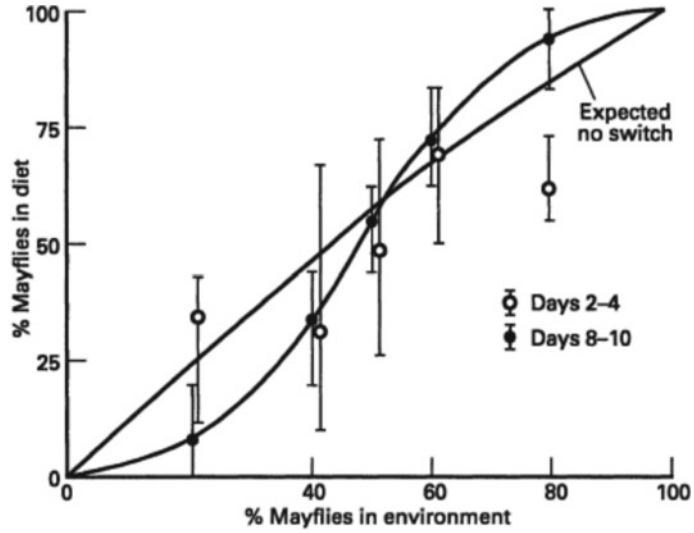


Fig. 1.20 Switching in insect natural enemies: the percentage of mayfly larvae in the diet of *Notonecta glauca* L. (Hemiptera: Heteroptera) was a function of their relative abundance in the habitat. The almost straight line is the 'no-switch' curve. *Source* Lawton et al. (1974)

of the most abundant prey species, i.e., they experience a perceptual change in the ability to detect a cryptic prey type, and this change does not occur when that type is rare (Lawrence & Allen, 1983; Guilford & Dawkins, 1987, discuss evidence for search image formation). However, switching could well result from other behaviour such as active rejection of the less preferred host species as the preferred hosts become more abundant, a prediction of optimal prey selection models (Sect. 1.6.7). Note that Murdoch's (1969) definition of switching is couched in terms of relative prey density, whereas optimal foraging models refer to absolute densities or encounter rates with prey.

Lawton et al. (1974) investigated whether experience with a particular prey species may be a contributory mechanism in the switching behaviour of *Notonecta glauca* presented with *Asellus* and *Cloeon*. In this case, negative switching was recorded over days 2–4 of the experiment and positive switching over days 8–10 (Fig. 1.20). In a separate experiment, the authors measured the proportion of successful attacks on *Asellus* prey in relation to the proportion of this prey available in the environment during the previous seven days. They found that the more *Asellus* the predator was exposed to, the greater was the proportion of successful attacks recorded. While this strongly suggests that experience with *Asellus* affects the predator's prey capture efficiency, it does not prove conclusively that it does so, since no information was obtained on the encounter rates, and therefore on the experience of the insects, during the pre-experimental period. The development of a search image could be ruled out as a mechanism for switching in this predator/prey system since: (1) in the switching test *N. glauca* took different prey species in a random sequence instead of attacking prey in 'runs' (Lawton et al., 1974), and (2) the prey were unlikely to have been cryptic in the experimental tanks used.

Switching behaviour may not necessarily be adaptive. Chow and Mackauer (1991) found that *A. ervi* and *P. pequodorum* switched to the alfalfa aphid when pea aphids and alfalfa aphids were offered to wasps in a 1:3 ratio. However, the authors hypothesised that since alfalfa aphids are more likely than pea aphids to escape from an attacking wasp, a foraging wasp incurs a potentially higher cost in lost 'opportunity time' (Sect. 1.6.7) when attacking alfalfa aphids. Furthermore, since it is possible that alfalfa aphids are poorer-quality hosts in terms of offspring growth and development, wasps may not derive a fitness gain from switching to alfalfa aphids.

1.13 Patch Defence Behaviour

When a predatory insect finds a resource, there is a trade-off between allocating time to consuming it or defending it against competitors (Field & Calbert, 1998). However, the resources utilised by parasitoids are relatively long-lived and thus potential hosts in a patch may not yet be suitable for attack, or any offspring that have already been invested in are vulnerable to attack themselves. As a result, some female parasitoids defend hosts, or patches of hosts, against conspecific and heterospecific intruders (Hardy et al., 2013; Couchoux & van Nouhuys, 2014; Mathiron et al., 2018; Goubault et al., 2019), occasionally leading to the death of one of the protagonists (Pérez-Lachaud et al., 2002; Velasco-Hernandez et al., 2021; Guo et al., 2023, see also Dunn et al., 2015). This patch defence behaviour consists of two components, resource defence (where competing females are prevented from gaining access to potential hosts; Waage, 1982) and maternal care (where previously parasitised hosts are protected from superparasitism or hyperparasitism; van Alphen & Visser, 1990), and the relative importance of both factors will influence patch defence behaviour (Field & Calbert, 1998; Guerra-Grenier et al., 2020). Thus, patch defence can be an alternative competitive strategy to one of allowing conspecifics on the same patch and competing with them through superparasitism (Sect. 1.9.4).

Patch defence is only advantageous under a limited set of conditions. The following factors favour defence of patches:

1. Synchronous development of the hosts in the patch.
2. Rapid development of the host to a stage which can no longer be attacked by the parasitoids, or rapid development of the parasitoid offspring to a stage at which they have a competitive advantage in cases of superparasitism (after which continued defence may no longer be necessary; Goubault et al., 2007b).
3. Short travel times between patches. When travel times are long, intruders are likely to be 'reluctant' to lose the contest for the patch. This would prolong fighting and increase the cost of defence.
4. A low probability of finding more than one host or host patch during adult life. This factor selects for foragers to spend long periods guarding those resources that they do find (host- and brood-guarding; Hardy & Blackburn, 1991).
5. Patches should be of a defensible size; larger patches are harder to defend.

Patch defence was first described for scelionid egg parasitoids (Waage, 1982), which defend small and intermediate-sized host egg masses. However, it is also found in braconids (e.g., *Asobara citri*), ichneumonids (e.g., *Rhyssa persuasoria*, *Venturia canescens*, *Hyposoter horticola*), and bethylids (e.g., *Goniozus nephantidis* and *G. legneri*).

In some parasitoids, such as the aforementioned scelionid egg parasitoids, patch defence appears to be a fixed response to an intruder (but see below). However, in other species such as the braconid *Asobara citri*, patch defence and fighting behaviour decrease in frequency with increasing patch size and increasing numbers of intruders, and wasps may switch to competition through superparasitism. Patch defence can have a pronounced effect on the distribution of adult parasitoids over host patches. It can lead to a regular distribution of parasitoids (de Jong et al., 2011), and is thus one of the factors reducing aggregation (Sect. 1.15).

Whether patch defence or competition by superparasitism is the better strategy depends on species-specific traits such as the encounter rate with hosts and the handling time. Thus, it is possible that one species attacking a certain host defends hosts or patches against intruders, while another parasitoid species attacking the same host does not.

One of the better-studied systems involves the scelionid wasp *Trissolcus basalis*, a parasitoid of the egg masses of pentatomid bugs (Field, 1998; Field et al., 1997, 1998; Field & Calbert, 1998, 1999; Sujii et al., 2002; Wajnberg et al., 2004; Cusumano et al., 2011; Mesterton-Gibbons et al., 2021; see also Guerra-Grenier et al., 2020). Here, if a female finds a patch, she will initially search for, and oviposit in, suitable hosts. Later, she will patrol the patch, still ovipositing until the patch is depleted. Once this occurs, the female will remain on the patch for about 5 h, before departing, although the length of time spent guarding will depend on patch quality. If two females find a patch, then at first both will exploit it without aggression. However, after a period of time, fighting will be initiated and the females take the roles of 'intruder' or 'resident', with the resident usually being the female that first arrived. The likelihood of a female *T. basalis* initiating conflict will depend on several factors: the number of potential hosts in the patch and the encounter rate with them, the asymmetry in arrival time and the number of conspecifics encountered, and the number of eggs invested in the patch. The resident will guard the patch because if the intruder attacks within the first 3 h after oviposition by the resident, the intruder's developing offspring will compete with those of the resident. During this period, the female will be exposed to a trade-off between exploiting new hosts and guarding the patch. The intruder will regularly attempt to cryptically invade the patch, and eventually will succeed once the resident has left. Finally, Wajnberg et al. (2004) found a significant intra-population genetic variation in the behavioural mechanisms involved in the patch defence strategy adopted by *T. basalis* females.

Patch defence should preferably be studied in multi-patch experiments. In single-patch experiments, one could easily underestimate the significance of defence behaviour. A classic example of this is the fighting and chasing which occurs when two females of *Venturia canescens* meet whilst searching the same patch. This aggressive behaviour is an important component of mutual interference in laboratory experiments with *V. canescens* (Hassell, 1978; Sect. 1.15.3). The function of the fighting and chasing is not easily understood from such experiments because the behaviour leads, on large patches, to a decrease in attack rate for both wasps but not to the permanent exclusion of the intruding wasp. However, field observations of *V. canescens* searching for *Ephestia* (*Anagasta*) *kuehniella* larvae feeding on fallen figs suggest that a fig containing a host larva can be successfully defended against intruding competitors, with the latter moving on to nearby figs following an aggressive encounter (Driessen et al., 1995). Field or semi-field condition observations are, of course, extremely valuable but often difficult to achieve and sample sizes may be in consequence relatively low (e.g., Couchoux & van Nouhuys, 2014).

1.14 Functional Responses

Understanding how predators and parasitoids respond to changes in prey and host density is critical to gaining a grasp of the interactions between natural enemies and their victims. Solomon (1949) coined the term functional response when describing the response shown by individual natural enemies to varying host (prey) density. With increasing host or prey availability, each natural enemy will attack more host or prey individuals, but several types of functional response are possible (Fig. 1.21, Chap. 7; Hassell, 2000b, provides a detailed review). Four types, called Type 1, 2, 3 and 4, have been observed:

Type 1: where there is a rectilinear rise to a maximum (N_x) in the number of prey eaten per predator as prey density increases. The response is described by the following equation:

$$N_a = a'TN \tag{1.3}$$

where N_a is the number of hosts parasitised or prey eaten, n is the number of hosts or prey provided, T is the total time available for search, and a' is an acceleration constant, the instantaneous attack rate (Eq. 1.3 applies only when $N < N_x$).

The Type 1 response is likely to be found when handling times (see below) are negligible and eggs are in limited supply.

Type 2: where the response rises at a constantly decreasing rate towards a maximum value, i.e., the response is curvilinear, in contrast with the Type 1 response. Holling (1959a, 1959b) predicted such a response, reasoning that the acts of quelling, killing, eating and digesting prey are time-consuming activities (collectively called the handling time) and that these will reduce the time available for further search. Following from this, as prey density increases, a predator will spend a decreasing proportion of its (total available) time on searching:

$$T_s = T - T_h N_a \tag{1.4}$$

where T_s is the actual time spent searching, and T_h is the handling time. The Type 2 functional response is probably the most commonly reported in parasitoids (Fernández-Arhex & Corley, 2003).

Type 3: where the response resembles the Type 2 response except that at lower prey densities it accelerates. The response is thus sigmoid.

Type 4: where the response resembles the Type 2 response except that at higher densities it declines, producing a dome-shape curve.

Sabelis (1992) also recognised a fifth type of response, which is intermediate between the Type 1 and the Type 2. This response appears to be shown by some predatory mites and will not be discussed further here.

The functional response of a predator or parasitoid species is usually measured as follows: individual insects are confined in an arena (e.g.,

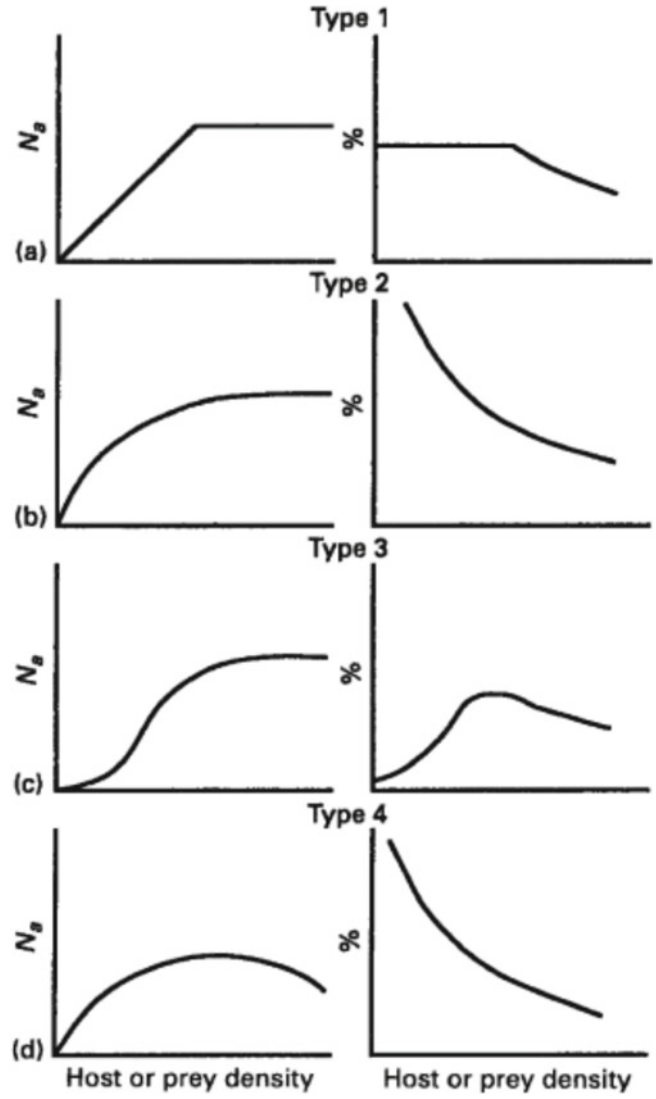


Fig. 1.21 The four types of functional response: **a** Type 1; **b** Type 2; **c** Type 3; **d** Type 4. N_a = number of hosts parasitised or number of prey eaten; % = percentage of hosts parasitised or eaten

cage), with different numbers of prey or hosts, for a fixed period of time (Fig. 1.22). At the end of the experiment, the natural enemies are removed and either the number of prey killed or the number of hosts parasitised (or both, in the case of some host-feeding parasitoids, see below) is counted. Hosts are either dissected or reared until emergence of the parasitoids. From the counts made, a graph can then be plotted relating the number of prey or hosts attacked to the number offered. The plots are then fitted to mathematical models (Holling, 1959a, 1959b, 1966; Rogers, Royama, 1971, 1972; Mills, 1982; Arditi, 1983; Casas et al., 1993; Casas & Hulliger, 1994; Dannon et al., 2010; Bodino et al., 2019; D'Auro et al., 2021; Wang et al., 2020; Aguirre et al., 2021).

Determining the type of functional response is an important step that needs to be taken by the investigator before attempting to obtain parameter estimates from functional response models. Incorrect estimates may be obtained if a model for a Type 2 response is used to estimate parameters from what is in reality a Type 3 response, and *vice versa*. For advice on how to determine the functional response, and for

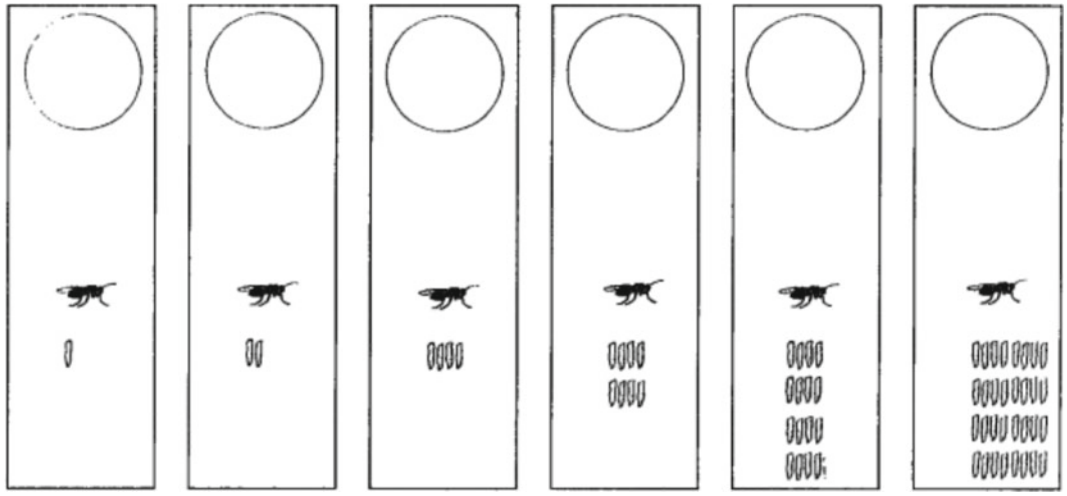


Fig. 1.22 Functional responses of parasitoids and predators: schematic representation of traditional design of a functional response experiment. Circles denote the experimental host patch, and rectangles the experimental arena. See text for discussion

information on curve-fitting routines, see Trexler et al. (1988), Casas and Hulliger (1994), Juliano (2001) and Schenk and Bacher (2002). There are R packages that can fit functional responses to experimental data uing correct statistical methods (e.g., frair, Pritchard et al., 2017). An alternative to measuring functional responses is to undertake an integrated analysis of all factors affecting patch time allocation in parasitoids (Sect. 1.5).

With predators, the functional responses of the different larval instars, as well as those of the adults, can be measured. With both predators and parasitoids, functional responses in relation to prey and hosts of different sizes can be measured.

There are two likely reasons why a Type 3 response may be recorded using the aforementioned experimental setup:

1. As host density decreases at the lower range of host densities, the parasitoid spends an increasing proportion of the total time available in non-searching activities. For example, at lower host densities, *Venturia canescens* spends a greater proportion of its time performing activities such as walking and resting on the sides of the experimental cage. Similar behaviour is probably responsible for the Type 3 response observed in parasitoids and predators that are offered unpreferred prey species: in *Aphidius uzbeckistanicus*, *Coccinella septempunctata* and *Notonecta glauca*, a Type 3 response was recorded when the unpreferred host and prey species was provided, compared with a Type 2 when the preferred species was provided (Hassell et al., 1977; Dransfield, 1979). The parasitoid under investigation may be a host feeder, mainly feeding upon hosts rather than ovipositing, at low host densities (Sect. 1.8) (in host-feeding parasitoids that feed and oviposit on different host individuals, we may distinguish between the following functional responses: that for parasitism alone, that for host feeding alone, and that for parasitism and feeding combined; i.e., the 'total' functional response; Kidd & Jervis, 1989). If, as is likely, the handling time for feeding encounters is longer than for oviposition encounters, a Type 3 response for parasitism may result (Collins et al., 1981).
2. Handling times may be shorter at higher host densities. In solitary parasitoids, this is an unlikely cause of a sigmoid functional response, but gregarious parasitoids may decrease clutch size at higher host densities (Sect. 1.10), and so decrease handling time per host. Predators may ingest less food from each

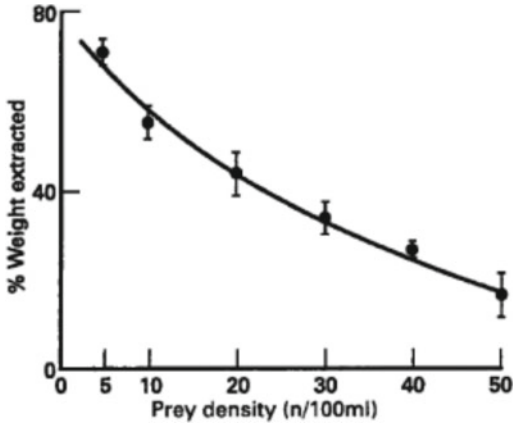


Fig. 1.23 The relationship between prey availability and the percentage of the mass of each prey individual consumed by the belostomatid bug *Diplonychus rusticum*: The bug is more 'wasteful', eating proportionately less of each prey (*Chironomus plumosus*) as prey density increases. This effect is predicted by optimal foraging (i.e., functional) and gut-filling (i.e., causal) models and is shown by a wide variety of predators. Values given are means ±SE. *Source* Dudgeon (1990)

prey item at higher prey densities (Fig. 1.23) and so reduce handling times. When extracting food from a prey item becomes increasingly difficult with the time spent feeding on it, predators may optimise the overall rate of food intake by consuming less of each individual prey item when the rate of encounters with prey is high (Charnov, 1976; Cook & Cockrell, 1978). Optimal foraging models predict this behaviour, while a similar prediction can be made based on a causal model relating the amount of food in the gut to the amount eaten from each prey. Some authors have argued that the optimal foraging model can be refuted because there is a causal explanation for the observed behaviour. However, causal and functional explanations are not mutually exclusive; indeed, they complement each other (Sect. 1.2). When predictions of a causal and a functional model are quantitatively similar, this can be taken as evidence that the mechanism does not constrain optimisation of a behavioural trait.

A Type 4 functional response will occur if: (1) when dealing with prey individuals, other prey individuals interfere with the predator and cause it to abort the attack more frequently at high prey densities than at low densities; and/or (2) the prey have a well-developed group defence reaction that is more effective at high prey densities than at low ones.

The classical functional response experiment assumes there is a homogeneous environment, or at least it does not consider the spatial distribution of prey and hosts. However, most insects are patchily distributed and the spatial distribution of hosts or prey within an experimental arena is likely to vary significantly with the density of the insects. Predators and parasitoids respond to differences in prey and host densities between patches by adjusting the amount of time spent in each patch (Sect. 1.5). By allowing the parasitoid, rather than the experimenter, to determine the amount of time it spends in an experimental patch (in a so-called variable-time experiment), a different type of functional response may be obtained compared to experiments where the time spent is fixed by the experimenter (so-called fixed-time experiments; Collins et al., 1981; van Alphen & Galis, 1983; Hertliein & Thorarinsson, 1987). Van Lenteren and Bakker (1978) suggest that in fixed-time experiments, some parasitoids are likely to show a Type 2 response, rather than a Type 3, because parasitoids are caused to revisit low-density patches they would otherwise leave. Thus, a Type 2 response may be an artefact of the fixed-time experimental design.

Designing an experiment for estimating functional responses from experimental data is usually considered a difficult task since the goal is to estimate the number of hosts or prey attacked as a function of the density of hosts or prey available. Such a density must remain constant (i.e., undepletable patches). Hence, any host or prey attacked should ideally be replaced immediately by a new one. Okuyama (2013) points at several methological problems like this one.

Since the type of functional response found in an experiment depends very much on the experimental design adopted, one should first clearly define what sort of question one wishes to address before measuring a functional response. Often, a functional response is measured to provide insights into the suitability of a parasitoid as a biological control agent. The problem is then how one can use the information generated by the experiments to predict the performance of the parasitoid in the field. The context in which the data will be used is one of population dynamics (D'Auro et al., 2021; Aguirre et al., 2021; Chap. 7), and thus relates to the response of the parasitoid population to host density. The spatial structure of natural host populations, and the interactions between individual parasitoids in the population, make it hard to relate the results of experiments on individuals in single-patch, single-parasitoid experiments to processes occurring at the population level.

If single-patch experiments are, nevertheless, to be carried out, the minimum requirements for experimental design should be as follows: the foraging insect should be observed continuously (in many functional response experiments, parasitoid and predator behaviour have not been examined directly) and a record made of how the parasitoid spends its time in the experimental arena. Parasitoids should be allowed to leave the arena when they decide to leave, so the experiment should be a variable-time one. It may prove difficult for the observer to decide when an experiment should be terminated. A parasitoid may leave the experimental patch for a short period, but then return and continue searching for hosts. Experiments may need to be terminated after the insect has spent an arbitrary period of time outside the patch (Waage, 1979; van Alphen & Galis, 1983), but of course the choice of the period is subjective and it acts as a censor in the data (a censor is a factor, other than a decision by the foraging insect, that terminates an experiment, e.g., a decision by the experimenter or an external disturbance, see Haccou et al., 1991; Sect. 2.8.2). A solution to the problem of when to terminate an experiment is to use an arena containing two patches. Once the insect has left the first patch and arrived in the second one, the experiment can be terminated.

However, there is a drawback to conducting such experiments under artificial conditions in the laboratory. The searching efficiency of the natural enemy will be influenced by the spatial structure of the patch (e.g., variation in plant architecture) and in the age structure of the victims (Wang et al., 2020). As an illustration of this, consider the parasitoid *Aphidius ervi* that preferentially attacks second- and third-instar pea aphids within a given patch. Among patches it exhibits variation in foraging efficiency resulting from variation in plant architecture. Ives et al. (1999) found that when aphid numbers were low *A. ervi*, a species that would normally be considered to exhibit a strong Type 2 functional response, shows a Type 1 response. Foraging experiments should therefore be conducted under a range of scenarios, of which at least some would reflect more natural foraging conditions.

Ideally, functional response experiments should measure encounter rates with concurrently available patches containing different densities of hosts. To do this, a multi-patch experiment needs to be carried out. Such an experiment might show that high-density patches are found more easily by the parasitoid, since such patches produce greater quantities of volatile attractants than low-density patches (Sect. 1.6.3).

Functional response experiments have typically not taken into account the possibility that the response of a parasitoid to patches of different densities depends on whether patches are scarce or common in the habitat. In 'poor' habitats when distances between patches are large and high-density patches are scarce, parasitoids should, when exploiting low-density patches, stay longer and parasitise more hosts. Finally, functional response experiments need to take account of the reaction of a parasitoid to the presence of conspecifics or other competitors (Aguirre et al., 2021).

1.15 Distribution of Parasitoids Over a Host Population

1.15.1 Introduction

The distribution of parasitoids over a spatially structured (i.e., heterogeneous) host population has attracted considerable attention from theoretical ecologists. Hassell and May (1973) and Murdoch and Oaten (1975), among others, have shown that this is one of the key features affecting stability of parasitoid–host population models (Chap. 7).

1.15.2 Aggregation

The term aggregation is usually used to refer to the host-searching behaviour of parasitoids. Parasitoids may be more attracted to patches of high host density than to patches of low host density or they may show a stronger degree of arrestment in patches of high host density (Sect. 1.6.3). Insect ecologists refer to an aggregative response of parasitoids and predators, because the aforementioned patch response behaviour leads to the concentration of parasitoids and predators in high-density patches (e.g., Vanbergen et al., 2007; Couchoux & van Nouhuys, 2014). Latterly, the term aggregation has also been applied to the concentration of parasitoids on patches of low host density or on certain patches irrespective of the number of hosts they contain; this can occur if parasitoids are attracted to some patches in response to stimuli that are either negatively correlated with, or independent of, host density. In studies of population dynamics, the term aggregation has also been used in a statistical sense, in terms of both the variance in parasitoid distribution and the covariance between the distributions of host and parasitoid (Godfray & Pacala, 1992).

Aggregation of adult parasitoids is the result of two different processes:

1. Differences among patches in the probability of discovery by parasitoids. In a heterogeneous environment, it is likely that not every patch has the same probability of being detected, even if all patches are otherwise similar. Patches may also differ in the probability of detection by parasitoids because of differences in host density or other aspects of quality of the patch.
2. The period of time that each parasitoid stays in a patch after discovering it. The number of parasitoids visiting a patch and the period of time they stay there determine the amount of 'search effort' devoted to a patch.

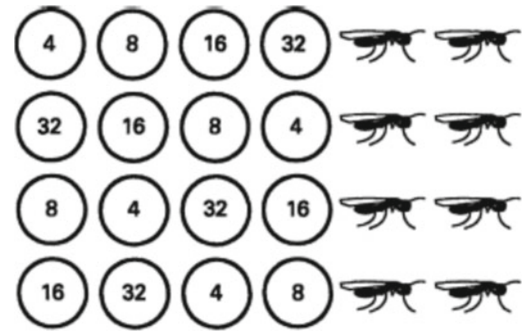


Fig. 1.24 Aggregative responses of parasitoids and predators: Schematic representation of the design for an experiment used for detecting and measuring the aggregative response, and which takes account of the effects on interactions between foragers. Numbers within circles indicate the number of hosts present in each patch

Aggregation can be measured in two main ways:

1. Individual parasitoids can be presented with several patches of different host density, as in studies of patch time allocation.
2. Several parasitoids at one time can be presented with several patches of different host density (Fig. 1.24).

When measuring aggregation using the second of these experimental designs, one should ideally monitor the behaviour of all parasitoids in all patches and record the time each parasitoid spends in each patch. In laboratory experiments with a modest number of host patches and with the insects continuously observed with video-recording equipment, this is possible, but in field experiments such observations are very labour

intensive and often impossible to make. Published field studies on aggregation have therefore relied on periodic observations of the patches (e.g., Waage, 1983; Cronin, 2003a).

One problem associated with studying aggregation in the field is deciding upon the spatial scale at which aggregation should be measured. Clumped distributions of hosts may occur at different levels of host distribution, and so may aggregation by parasitoids (e.g., Doak, 2000). It is often possible, for practical purposes, to define what a patch is. For example, when studying the distribution of parasitoids of the cassava mealybug within a cassava field, cassava plant tips infested with mealybugs are the most relevant foraging units, whereas if one wants to compare biological control between different fields, whole cassava fields can be considered as patches.

The dispersal behaviour of the predator or parasitoid itself may also influence the aggregation pattern of aggregation seen. The likelihood of the minute fairyfly (Mymaridae) egg parasitoid *Anagrus sophiae* laying all of its eggs is correlated with dispersal distance among patches. Females that have dispersed over 250 m from their natal patch will oviposit all their eggs in that patch (Cronin & Strong, 1999).

Such patterns may also be environment-dependent. Cronin (2003b) found that parasitism of the planthopper *Prokelisia crocea* by the egg parasitoid *Anagrus columbi* depended on the location of the planthopper's host plant, prairie cordgrass. When plant patches were surrounded by other grass species, parasitism rates were lower on the periphery of the patch, whereas if the host plants were surrounded by mudflat, attack rates were even throughout the patch. The dispersal behaviour of the parasitoid also varied with cordgrass patches surrounded by non-host grasses having a higher likelihood of colonisation by the egg parasitoid. It is evident that, ideally, studies of parasitoid aggregations should combine knowledge of host, and host-plant, distributions with an understanding of parasitoid dispersal and foraging behaviour (Vanbergen et al., 2007).

1.15.3 Interference

Before we describe interference, we need to stress that mutual interference, pseudo-interference and indirect mutual interference are concepts that can only be properly understood with reference to mathematical models, in particular those of searching efficiency (Chap. 7). The reader is therefore recommended to explore literature dealing with host–parasitoid population dynamics (e.g., Hassell, 2000a, 2000b).

The tendency for some parasitoids and predators to cease searching and to leave the immediate vicinity after an encounter with a conspecific would account for the results of laboratory experiments designed to measure emigration rates in relation to parasitoid density. In these experiments, the proportion of female parasitoids leaving a single, fixed-density host patch increased significantly with increasing numbers of parasitoids (see Fig. 1.25 for experimental design). It has also been observed that when females encounter either an already parasitised host or a parasitoid mark on the substratum, they move away from the area where the encounter occurred. Any of these behavioural interactions are likely to cause the searching efficiency of a natural enemy in the single-patch experiment to be reduced, a phenomenon known as mutual interference (Hassell & Varley, 1969; Visser & Driessen, 1991; Lynch, 1998; Kristoffersen et al., 2001; Elliott, 2003; Couchoux & van Nouhuys, 2014; Yazdani & Keller, 2015; Sreenivas & Hardy, 2016).

The study of mutual interference began with Hassell and Varley (1969) who noted a negative relationship between parasitoid searching efficiency and the density of searching parasitoids:

$$\log a' = \log a - m \log P \qquad (1.5)$$

where P is the density of searching parasitoids; a' is the effective attack rate or area of discovery per generation, $a'P$ = log [initial number of hosts/number of hosts surviving parasitism]; a is the attack rate in the absence of interference. The

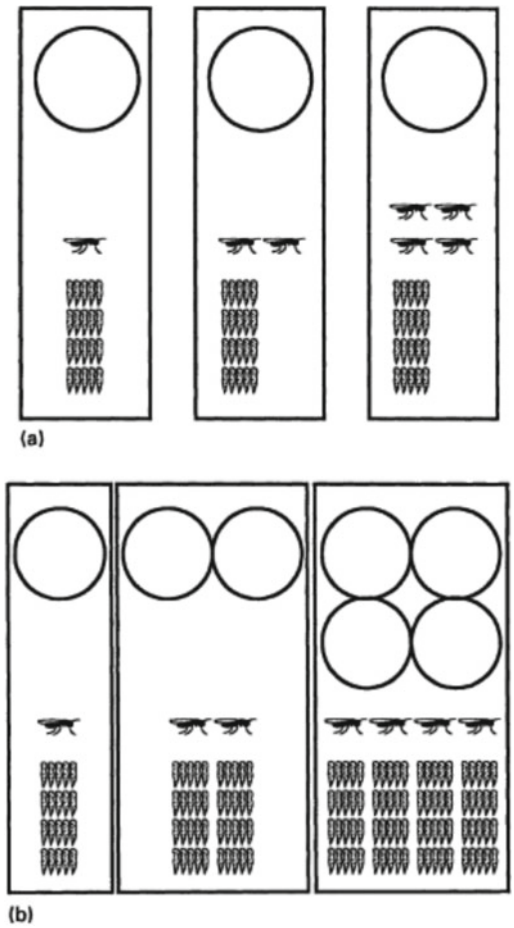


Fig. 1.25 Mutual interference and pseudo-interference: Schematic representation of the design of two types of experiment for studying interference: **a** the design normally adopted for measuring interference, with a single host patch (denoted by a circle); **b** the design used by Visser et al. (1990): either a single parasitoid female searches a single unit patch containing 20 hosts, or two females search a double unit patch containing 40 hosts, or four females search a quadruple unit patch containing 80 hosts. Hassell (1971a, 1971b) used a design similar to that given in Fig. 1.22, although the number of foraging parasitoids was also varied. Both Visser et al. (1990) and Hassell (1971a, 1971b) designs take account of the multi-patch context in which interference occurs

parameter m is the measure of the extent of mutual interference. Such a relationship is to be expected because, as parasitoid density increases, individual parasitoids will waste an increasing proportion of their searching time in encounters with other conspecifics. Similar patterns are to be found with insect predators, and even among closely related species the importance of mutual interference will vary (Elliott, 2003).

Free et al. (1977) argued, using deductive models, that marked parasitoid aggregation (e.g., resulting from a strong tendency of parasitoid individuals to spend longer periods of time in higher host-density patches, and the consequent differential exploitation of patches) can lead to apparent interference, termed pseudo-interference, even if behavioural interference is lacking. As a consequence of parasitoids aggregating in high-density regions (because these are initially the most profitable), a higher proportion of the hosts in the whole area (i.e., experimental cage) is parasitised than would be obtained with random search. If parasitoids do not respond (i.e., by dispersal) rapidly to the declining profitability of the high host density (i.e., more heavily exploited patches), then overall searching efficiency will be lower at high parasitoid densities. Thus, pseudo-interference results from 'over-aggregation' by the parasitoids (Hassell, 1982). In a population of optimally foraging parasitoids capable of responding rapidly to exploitation, overall searching efficiency would, at high parasitoid densities, be the same as for random search.

A third form of interference has been identified (Visser & Dreissen, 1991; Visser et al., 1999). Indirect mutual interference was first found in the parasitoid *Leptopilina heterotoma*, a generalist parasitoid of drosophilids. Mutual interference is not found in this species, but as a result of superparasitism, searching efficiency is reduced at the population level, but not at the the level of the patch (Visser & Dreissen, 1991).

These different forms of interference can lead to the stabilisation of consumer–victim population interactions, and as a result have proved important in studies linking individual behaviour and population dynamics. Visser et al. (1999) used data collected by Jones (1986), studying *Trybliographa rapae* attacking *Delia radicum*, to explore how the three different forms of interference may influence host–parasitoid population dynamics. In this case, the effect of interference depended on host distribution and the parasitoid's arrival and departure rules. Mutual interference did not appear to be important, but

both indirect mutual interference and pseudo-interference reduced parasitoid search rate, their relative importance depending upon host distribution.

Traditionally, parasitoid attack rates (number of hosts parasitised per unit time) are used when considering interference relationships. However, if one is concerned with optimal behaviour, encounter rates should be considered. Visser et al. (1990) and van Dijken and van Alphen (1991) went further and calculated the mean number of realised offspring per female parasitoid per unit of patch time as a measure of individual efficiency. ESS models developed by Visser et al. (1992a) predict that the presence of other females on a patch reduces this efficiency, even when the number of hosts per female is held constant. This interference is not caused by behavioural encounters that decrease the encounter rate with hosts but results from the parasitoids staying for longer on patches and superparasitising.

It needs to be stressed that when investigating interference phenomena, the parasitoid densities used and the host spatial distribution pattern should typically reflect those found in the field. As pointed out by Free et al. (1977), few experimenters take account of this requirement. Nonetheless, deliberately exposing parasitoids to unusual densities can elict behaviours and aspects of reproductive performance that generate useful insights (e.g., Venkatesan et al., 2009; Sreenivas & Hardy, 2016; Abdi et al., 2020a; Malabusini et al., 2022).

Another factor to consider is the size of the experimental arena. Jones and Hassell (1988) found *per capita* searching efficiencies to be lower in field cages than in laboratory cages and interference to be more marked in the latter, the volume of which was relatively small. Jones and Hassell (1988) attributed the interference to an unnaturally high frequency of encounters between searching parasitoids (*Trybliographa rapae*). The much lower searching efficiency in the field cages was presumably due to the greater opportunities for parasitoids to spend time performing behaviour other than searching in close proximity to hosts.

It should also be noted that not all interactions between foraging parasitoids are necessarily negative. For example, if parasitoids respond to the presence of others by reducing handling time or by avoiding areas previously searched by conspecifics (through patch marking), then 'positive interference' may occur (Visser et al., 1999); inded some parasitoids may even cooperate in host attack (Abdi et al., 2020b, 2020c; Liu et al., 2021).

1.16 Life-History Traits and Foraging Behaviour

1.16.1 Introduction

Insect parasitoids display an enormous diversity of life-histories (Blackburn, 1991a, 1991b; Godfray, 1994; Quicke, 1997; Mayhew & Blackburn, 1999; Jervis et al., 2001, 2003; Traynor & Mayhew, 2005; Jervis & Ferns, 2011; Iwabuchi, 2019; Poelman et al., 2022; Hardy & Godfray, 2023; Chap. 2). Some species have very short development times, can live for only a few days as adults, and emerge with all their eggs ready to be laid, in contrast to other species which develop slowly, can live for several months as adults, and produce new eggs throughout adult life. Some species produce a large number of small eggs, whereas others produce a small number of large eggs. These different life-history traits are associated with differences in searching and host selection behaviour. When designing experiments on parasitoid behaviour, it is important to be aware that this is so. We discuss this in relation to egg production strategy.

1.16.2 Egg Limitation *Versus* Time Limitation

Given that eggs are costly to produce and allocation to reproductive function will trade off with longevity (Segoli & Wajnberg, 2020, but see Segoli et al., 2018), natural selection can be expected to lead to reproductive strategies that

approach a quantitative match between egg supply and the availability of suitable hosts. An evolved match may become a mismatch if, for instance, host populations gradually decline over ecological time (Couchoux & van Nouhuys, 2014) or mismatches may be due to within-season variation in host availability (Phillips & Kean, 2017). More generally, aspests of environmental variability will typically prevent exact matches, leading to females either running out of eggs before death (egg limitation) or dying with eggs unlaid (time limitation). Whether egg limitation or time limitation is predominant has been extensively debated. The consensus is that either may occur, depending on the details of the environmental variability (Rosenheim, 2011; Phillips & Kean, 2017).

Parasitoids can be divided into pro-ovigenic and synovigenic species (Sect. 2.3.4). Pro-ovigenic parasitoids emerge with their full potential lifetime complement of mature eggs, whereas synovigenic parasitoids emerge with at most only part of their complement, this fraction varying considerably among synovigenic species (ranging from very nearly one down to zero) (Boivin & Ellers, 2016; Jervis et al., 2001). These different patterns of egg production can be understood as adaptations to differences in the spatial and temporal distribution patterns of hosts (Jervis et al., 2001; Ellers & Jervis, 2003). Pro-ovigenic species are expected to behave in laboratory experiments in a time-limited manner, because even when large numbers of hosts are offered, these numbers do not exceed the number of mature eggs carried by the parasitoid. Conversely, synovigenic species are expected to behave in an egg-limited manner, often exhausting their daily egg supply in a few hours when the number of hosts offered to them exceeds the number of mature eggs in their ovaries (this oversimplifies the difference between pro- and synovigeny; see Ellers et al., 2000, for further details).

Theoretical studies considering stochasticity in host availability have shown it to be a major influence on optimal egg loads, and that the patchy distribution of hosts is a key source of stochasticity (Rosenheim, 1996; Ellers et al., 1998, 2000; Ellers & Jervis, 2004; 2011), as is temporal stochasticity in reproductive opportunities (Rosenheim, 2011; Phillips & Kean, 2017). If stochasticity is high, investment is shifted away from lifespan to eggs, i.e., towards an optimal egg load that is higher than the expected number of hosts found, and thus a lower incidence of egg limitation. The ability of synovigenic parasitoids to mature eggs throughout life further reduces the incidence of egg limitation and it also reduces the degree to which individuals are time limited (i.e., they have a surplus of eggs but not too many) (Ellers et al., 2000). However, synovigenic females will still experience transient levels of egg limitation (Heimpel & Rosenheim, 1998; Heimpel et al., 1998; Casas et al., 2000; Rosenheim et al., 2000). Further, egg limitation may be ecologically important even when not prevalent, and the population dynamics of the hosts are also likely to influence selection for egg investment strategies (Phillips & Kean, 2017).

From a literature survey of fifteen species, Heimpel and Rosenheim (1998) concluded that egg limitation is common in the field. The results of empirical field studies, however, suggest that only some females experience egg limitation (Weisser et al., 1997; Ellers et al., 1998; Heimpel et al., 1998; Casas et al., 2000; Phillips & Kean, 2017; Segoli & Rosenheim, 2013b). Thus, it appears that parasitoids have evolved strategies that reduce the risk of egg limitation. However, concomitant with these would be an increased risk of time limitation, the risk being heightened by any lifespan cost, of egg production (Ellers et al., 2000). Indeed, West and Rivero (2000), using a sex ratio-based method to measure the relative importance of egg and time limitation among eight parasitoid species, concluded that on average, most species are at an intermediate position along the egg/time limitation continuum, with a bias towards time limitation.

The question of whether time and egg limitation, when observed in the laboratory, reflect the field situation or whether it is an artefact of unnaturally high host densities can be addressed by obtaining some measure of oviposition rate under field conditions, and comparing this with

the average rate of egg production in parasitoids. The outcome of experiments on aspects of parasitoid biology as diverse as patch time allocation, functional responses, host selection, sex allocation, superparasitism or encounter rates, will all depend critically on whether the experimental conditions place the parasitoid under the constraint of time or egg limitation. Either experiments can be run under conditions representing both of these constraints, or an experimental design can be chosen that is relevant to the particular question one is asking. For example, when asking about the performance of a parasitoid immediately following field release, present females in experiments with a superabundance of hosts so that they are egg limited, but when asking about the performance of the parasitoid after the host population has been suppressed below a damage threshold, present females with low densities of hosts so that the parasitoids are time limited. If one is asking evolutionary questions, it is advisable to choose a situation (e.g., range of host densities, host spatial distribution pattern) closest to what the wasps experience most often in nature.

1.17 The Cost of Reproduction

In many studies of time allocation, recognition time and handling time are taken to be the only time costs involved in oviposition. However, as discussed in Chap. 2, a trade-off can exist between reproductive effort and survival (e.g., Thorne et al., 2006). In at least one case, it appears that egg deposition, as opposed to egg production, incurs a survival cost (Sect. 2.8.3).

1.18 Age-Dependent Foraging Decisions

Although parasitoids may have a longer life expectancy when they lay fewer eggs, they do not live forever. The older they become, the less likely they are to survive to another day (e.g., Hardy et al., 1992b; Amante et al., 2017; Jucker et al., 2020). In addition, young adult parasitoids may be more fecund than older females (De Vis et al., 2002; Riddick, 2003). Because of the diminishing probability of survival with increasing age, parasitoids should become less selective and accept more host types for oviposition (Iwasa et al., 1984). For example, young *Lysiphlebus cardui* preferentially attack second- and third-instar *Aphis fabae*, whereas older wasps show no preference (Weisser, 1994). All other things being equal, older wasps will superparasitise and accept less suitable hosts more readily than younger ones, a prediction that is supported empirically (Roitberg et al., 1992, 1993). One can try to make use of this alteration in behaviour with age in experiments that require parasitoids to oviposit in non-preferred hosts, e.g., parasitised individuals and unpreferred species, or to explore how the subjective value of hosts affects patch defence behaviour (Humphries et al., 2006; Stockermans & Hardy, 2013; Sect. 1.13).

1.19 Foraging Behaviour and Taxonomy

Taxonomists work primarily with preserved specimens and until recently relied heavily on morphological characters to describe species (Gauld & Bolton, 1988; Quicke, 1993). This is in most cases a satisfactory state of affairs, because differences in morphology can often be found, even between closely related species. Sometimes, however, morphologically identical specimens can be collected from populations found in ecologically different situations, e.g., attacking a different host species, occurring on different host plants or in different geographical regions. The question then is whether these populations belong to one species or not: an important question, not only in deciding whether a parasitoid is a specific natural enemy of a target pest, but also because the scientific name of an organism is used in publications.

By comparing the host habitat-finding behaviour and host selection behaviour of different populations, one can establish whether important ecological differences exist between them. Differences in host habitat finding and/or host

species selection can theoretically result in reproductive isolation between the two populations, which occupy different niches by virtue of the differences in their searching behaviour. When interpopulation differences in foraging behaviour are found, one should then determine whether cross-matings are possible. If such matings do not occur either in the laboratory or in the field, it is reasonable to conclude that the populations are separate species.

Vet et al. (1984) discovered *Asobara rufescens* by studying microhabitat location of wasps initially believed to be *A. tabida. Asobara rufescens* had until then gone unrecognised and its populations had been considered conspecific with *A. tabida*. Similarly, van Alphen (1980) discovered a new species of *Tetrastichus*, which attacks the twelve-spotted asparagus beetle, *Crioceris duodecimpunctatum*, by showing that it rejected the eggs of *Crioceris asparagi*, the host *of Tetrastichus coeruleus*. Information about the foraging behaviour of insect natural enemies may therefore prove useful in taxonomy and systematics, although this is complicated by behavioural plasticity (Japyassu & Viera, 2002).

1.20 Foraging Behaviour and Host Resistance

1.20.1 Introduction

Not all prey or host individuals are equally worth attacking. It has become increasingly clear that the success rate of natural enemy attack can vary due to host or prey defence. Such resistance may take many forms, but this can be conveniently divided into physiological and behavioural defences.

1.20.2 Physiological Host Resistance

Physiological defences to endoparasitoid attack centre on the innate immune response of insects, which typically involves the parasitoid egg being isolated in a melanised capsule (Sect. 2.10.2); a counter-strategy by parastioids may be to lay multiple eggs into the host such that they cannot all be encapsulated (van Alphen & Visser, 1990; Luna et al., 2016; D'Auro et al., 2021). The immune response is not, however, the only means by which hosts avoid the detrimental actions of parasitoids: many herbivorous insects sequester plant secondary chemicals that can be deployed as a means of defence against their natural enemies. *Utetheisa ornatrix*, an arctiid moth, feeds on legumes from which it sequesters pyrrolizidine alkaloids (Eisner et al., 2000). These alkaloids are passed onto the eggs, and this acts as a deterrent against the predatory lacewing, *Ceraeochrysa cubana*. However, the amount of alkaloid passed down to the eggs varies, depending on the host plant the parents have been feeding on. The moth's eggs are laid in batches of about twenty, and the lacewing will sample two or three before deciding to accept or reject the batch of eggs. Since the variation in noxious chemicals within a batch is low, sampling from a small number will provide a reliable indicator of prey quality. If there is considerable variation among batches in alkaloid concentration, sampling from all batches is worthwhile (Eisner et al., 2000).

It has been suggested that such secondary chemicals are more likely to be sequestered by specialist herbivores than by generalists. This leads to the prediction that generalists should be subject to greater levels of attack by natural enemies, and that levels of attack should reflect the presence of the secondary chemicals in the community. In an elegant experiment, Camara (1997) tested the latter hypothesis under natural conditions. Buckeye butterfly larvae, *Junonia coenia*, were reared on plants that contained iridoid glycosides (*Kickxia elatine* and *Plantago lanceolata*) or an artificial medium lacking the defensive chemicals. In the sites where many plants contained iridoid glycosides, fewer larvae that had been fed on the plants were consumed by predators, whereas no differences in predation were found in sites with lower proportions of the glycoside-containing plants (Camara, 1997). As well as individuals varying in the amount of secondary chemicals acquired from host plants, substantial variation among populations is also likely.

1.20.3 Behavioural Defences

Perhaps the classic example of a behavioural defence against predator attack is provided by aphid dropping behaviour (Losey & Denno, 1998a, 1998b, 1998c). Here, aphids drop from the plant in response to predator cues, although this may not always be to the benefit of the aphid, as ground predators will often successfully attack the escapees (Losey & Denno, 1998c). Pea aphids (*Acyrthosiphon pisum*) show genetic variation in dropping behaviour, and this behaviour is influenced by ambient temperature (Stacey & Fellowes, 2002). Aphids can also escape predation by the production of winged morphs in response to the presence of predators (Weisser et al., 1999).

For aposematic prey species, i.e., those with colouration or markings that repel or warn predators of their unsuitability as prey, both physiological and behavioural defences may be intertwined. Tullberg et al. (2000) showed that while two species of lygaeid bugs (*Lygaeus equestris* and *Tropidothorax leucopterus*) are unpalatable to birds, the likelihood of being preyed upon was in part determined by the degree of aggregation of the larvae. Fewer attacks occurred when the larvae were in groups, compared to individual larvae.

Some hosts are similarly able to resist parasitioids by dropping from the host plant (aphids) or jumpig away (leafhoppers and planthoppers) and others may aggressively defend themselves (reviewed by Gross, 1993). Dipteran, lepidopteran and coleopteran larvae may wriggle, or otherwise defend themselves. Mealybugs may 'flip' the posterior end of their body or throw droplets of honeydew onto parasitoids attempting to parasitise them. Females in the ectoparasitoid genus *Sclerodermus* attack the larvae of wood-boring cerambycid beetles and face a mortality risk of around 20% when attacking small hosts and considerably higher risks when hosts are larger (Liu et al., 2011; Wei et al., 2014; Abdi et al., 2020b, 2020c). Host larvae react violently when attacked and have well-developed mandibles, and often the attacking wasp is bitten in two before it is able to subdue the host with an injection of venom (Abdi et al., 2020b, 2020c; Liu et al., 2021). The dangers involved in attempting to subdue such hosts are expected to influence the degree of cooperation and competition exhibited by *Sclerodermus* females which, if hosts are successfully suppressed, will go on to produce a communal brood (Tang et al., 2014; Mesterton-Gibbons & Hardy, 2021; Liu et al., 2021).

Irrespective of the means of avoiding or resting attack, it is clear that not all hosts are equal in value to a foraging predator or parasitoid. They vary not only in quality as a resource, but also in terms of the likelihood of their being successfully attacked, and this will vary among individuals, populations and species. Additionally, although much less studied, it is clear that there will also be variation at a similar series of scales in natural enemy 'virulence'.

1.21 Insect Natural Enemy Foraging Behaviour and Community Ecology

The role of insect natural enemy foraging behaviour in determining community interactions has been implicitly rather than explicitly implicated in many aspects of community ecology. These issues are well illustrated by the work of Müller et al. (1999), who studied an aphid-natural enemy (primarily parasitoids) system in Rush Meadow, an abandoned field in southwestern England. By producing a quantitative food web of the interacting species, Müller et al. (1999) were able to use the web to predict the strength of both direct and indirect interactions within the community. Such webs are immensely time-consuming to obtain but the return, measured in terms of detailed knowledge of the system, is potentially enormous (e.g., Schönrogge & Crawley, 2000; Lewis et al., 2002; Sect. 6.3.12). This web can be used to convincingly demonstrate the concept of apparent competition, where two species that do not directly compete for resources indirectly compete because of shared natural enemies (Holt, 1977; Sect. 7.3.7). Müller and Godfray (1997) tested for apparent

competition between the grass aphid, *Rhopalosiphum padi*, and the nettle aphid, *Microlophium carnosum*, mediated by shared natural enemies. They found that foraging ladybirds were attracted in increased numbers to the experimental site, mainly by the presence of grass aphids. However, the increased numbers of coccinellids preferentially attacked the nettle aphids, providing a clear example of apparent competition (Müller & Godfray, 1997).

Such food webs not only illustrate the importance of indirect effects within a community, but also point to the direct influence of insect natural enemies on community structure. Müller and Godfray (1999) studied why two species of aphids (*Aphis jacobaeae* and *Brachycaudus cardui*), common in surrounding areas, were uncommon in Rush Meadow. By excluding predators from artificially inoculated aphid colonies, they found that the aphids were able to colonise the field but were prevented from doing so by the presence of predators and parasitoids.

Clearly, the foraging decisions and abilities of insect natural enemies will have a significant influence on the community dynamics of any terrestrial ecosystem. Foraging success will often be influenced by the host plant a potential prey or host is attacking. For example, in *Encarsia formosa* (a parasitoid of whitefly), foraging success is greater on glabrous (smooth-leaved) varieties of cucumber (Hulspas-Jordaan & van Lenteren, 1978). In a different field system, recent field studies showed that the sizes and architectures of two plant species fed on by a single host species affects the species of parasitoid that attacks it, such that resource partitioning, parasitoid coexistence, and thus a more complex web, are likely promoted (Xi et al., 2017, 2020). Indirect effects of plants will also be common, as host species reared on poor-quality plants will have reduced population growth rates, and parasitoids emerging from such hosts may be smaller and less fecund (Stadler & Mackauer, 1996), while predators may consume more prey individuals for the same return.

Quantitative food webs also illustrate another important facet of insect natural enemy community ecology. While many predators and parasitoids are thought to have an extremely wide host range (the fundamental niche), these ignore the effects of host preference or competition, which results in a much narrower range of regularly attacked victims (the realised niche).

From the above examples, it is readily apparent that the foraging behaviour of insect natural enemies is often (within bounds) context-specific, and the range of species attacked will depend on both direct and indirect effects within the community. Moreover, the strength of these interactions may be influenced by the herbivore's host plants. Without an understanding of the natural history of the species of choice, designing laboratory-based systems to assay behaviour is fraught with difficulty. The importance of the community context in trying to understand the foraging behaviour of an insect predator or parasitoid should not be underestimated.

1.22 Concluding Remarks

The study of the foraging behaviour of insect predators and, especially, parasitoids has provided a model system for ecologists for many years. Applied ecologists use such systems in the hope that information obtained from them will inform biological control measures (Wajnberg et al., 2016; Heimpel & Mills, 2017) and link to population dynamics (Godfray & Shimada, 1999; Hassell, 2000b; Segoli et al., 2023; Chap. 7).

Recent decades have seen rapid advances in the study of insect natural enemies, particularly as regards parasitoid behaviour. These developments have mainly been in response to advances in ecological theory. The availability of a whole suite of models of parasitoid–host population dynamics that incorporate important behavioural characteristics of parasitoids, combined with the rapid progress in behavioural ecology, has led to the formulation of more precise and quantitative hypotheses. In addition, tools for the analysis of complex time-series of behaviour have become available, allowing us to address problems that previously could not be analysed properly.

Behavioural studies of parasitoids have been conducted along two main lines: the functional

analysis of behaviour, which has been guided by largely model-based theories on the evolution of animal behaviour, and the causal analysis of behaviour, which has been guided much less by models (Chap. 4). Developments in causal and functional analyses of behaviour have been, for a large part, independent. As we hope to have indicated in this chapter, research can benefit from an increased integration of the study of mechanisms and the study of the function of behaviour.

There is a productive, and indeed synergistic, two-way interaction between theory and empirical research: behavioural and population models can guide us in the design of experiments, while the results of experiments stimulate new theory. Theories of population dynamics and behavioural ecology are now often concerned with the behaviour of parasitoids and predators in spatially heterogeneous environments, with patchily distributed resources. For reasons of convenience, behavioural studies on natural enemies have often been conducted in single-patch environments. Results from experimental studies on the behaviour of natural enemies in multi-patch environments, preferably in natural settings (Heimpel & Casas, 2008), can provide the information needed both to test current theories and to develop new ones.

Along with this call for a meshing of empirical and theoretical ecology is the continuing need for researchers to have a firm grasp of the natural history of the species they are working with. Natural history is difficult, if not impossible, to learn in the lecture theatre or the laboratory, and there is no better way of beginning to understand the ecology of any system than spending time with it in the field. Becoming thoroughly acquainted with one's study organisms must be a prerequisite for any successful research programme.

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The Life-Cycle

2

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2.1 Introduction

This chapter is concerned with approaches and techniques used in studying those aspects of parasitoid and predator life-cycles that are relevant to the topics covered by other chapters in this book. To illustrate what we mean, consider the female reproductive system of parasitoids, discussed in some detail in Sect. 2.3. As pointed out by Donaldson and Walter (1988), at least some knowledge of its function, in particular of the dynamics of egg production, is crucial to a proper understanding of foraging behaviour in parasitoids. The state of the ovaries may determine: (1) the duration of any pre-oviposition period following eclosion; (2) the rate of oviposition, (3) the frequency and duration of non-ovipositional activities, e.g., host-feeding, and (4) the insect's response to external stimuli, e.g., odours, hosts (Collins & Dixon, 1986) (Sect. 1.5.1). Note that egg load (defined in Sect. 1.2.2) is now often incorporated into foraging models, as it has become clear that key foraging decisions depend upon the insect's reproductive state (Jervis & Kidd, 1986; Mangel, 1989; Chan & Godfray, 1993; Heimpel & Rosenheim, 1995; van Baalen, 2000; Heimpel & Casas, 2008). It also follows from the above that a female parasitoid's searching efficiency depends upon the functioning of its reproductive system and this may in turn influence parasitoid and host population processes (Chap. 7).

Comparative studies have provided useful insights into the factors that determine patterns of cross-species variation in the life-history traits of parasitoids, predators and spiders. The results of these investigations are only touched upon in this chapter; for further details, see Blackburn (1991a, b), Gilbert and Jervis (1998), Mayhew and Blackburn (1999), Strand (2000), Jervis et al. (2001, 2003), Mayhew and Glaizot (2001), Traynor and Mayhew (2005) and Mayhew (2016) on parasitoids, Dixon (2000, and

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references cited therein) on predatory coccinellids and Prenter et al. (1999); Lowe et al. (2020); Macías-Hernández et al. (2020). Godfray (1994) and Quicke (1997) should also be consulted for information on comparative aspects of parasitoid biology.

Much of the chapter is devoted to methods of recording variation in key life-history traits. Investigators should be mindful of the potential for trade-offs to occur between life-history variables, as predicted by general life-history theory (e.g., Roff, 2002). Examples of phenotypic trade-offs are given in the various sections on fecundity, adult longevity, development and growth and immature survival. Partly because of such trade-offs, caution should be exercised in using individual life-history traits as proxy measures of fitness (Roitberg et al., 2001). Genetic aspects of trade-offs are discussed in Chap. 3.

2.2 Anatomical Studies on Natural Enemies

2.2.1 Introduction

A general introduction to insect structure and function can be found in most standard entomological texts, e.g., Wigglesworth (1972), Chapman (1998, 2013), Richards and Davies (1977), Commonwealth Scientific and Industrial Research Organisation (1991). Individual topics are covered in texts such as Snodgrass (1935) on morphology, and Engelmann (1970) and Kerkut and Gilbert (1985) on insect reproduction. There are also texts such as Hodek (1973), Gauld and Bolton (1988), Quicke (1997), McEwen et al. (2001), Quicke (2014) and Ramírez and Michalik (2019) that deal with aspects of the anatomy and morphology of particular taxonomic groups of insect natural enemy. This section is concerned with methods used for investigating the internal anatomy of predators and parasitoids, the emphasis being placed on the female reproductive system.

2.2.2 Techniques

2.2.2.1 Dissection

Many insect natural enemies, particularly parasitoids, are so small that routine investigations of their internal anatomy might, at first sight, seem impossible to undertake. One approach to anatomical investigation is to fix and then embed insects in wax or resins, and then to cut, using a microtome, serial sections of the body. This method is, however, technically difficult and there usually arise problems such as distortion (e.g., due to hardness of the cuticle), inadequate fixation and the difficulty of reconstructing sections into a three-dimensional model. A far easier approach is to dissect the insect.

In order to carry out dissection, the following equipment will be required: a stereomicroscope with incident lighting (preferably fibre optics, see below), ordinary or cavity microscope slides, insect saline (e.g., 7.5 g NaCl/L) and some fine pins. The latter are best securely mounted either in glass tubes (4 mm diameter and approx. 50 mm long) or in matchsticks.

For parasitoid wasps (Trichogrammatidae, Mymaridae and others up to 25 mm long), place one droplet of insect saline on to a microscope slide and place the insect in the droplet. Use insects that have been recently killed either with ether, carbon dioxide, with some other suitable killing agent, or by freezing. Individuals that have been dead for more than an hour at room temperature, and also those that have been preserved in alcohol, are very difficult to dissect, so storing insects in a deep freeze is highly recommended. When dissecting, ensure that the insect's body is dorsal side up, feet down. With one pin, restrain the insect from floating or otherwise moving in the saline, either by piercing its thorax, or by holding the pin across the female's petiole. With the second pin, make small lateral incisions in the distal part of the gaster, preferably where there is an intersegmental membrane. Place the point of the second pin firmly upon the tip of the insect's gaster and

pull the latter gently away from the remainder of the gaster. The abdominal wall should part in the region of the incisions, and the abdominal contents should then spill out into the saline droplet. With a little practice, this technique will permit examination of the entire reproductive system, and also of the mid and hind gut. By carefully noting the positions of all the various organs during dissection, it should be possible to reconstruct the spatial arrangement of the organs and associated structures (Fig. 2.1). More difficult manipulation may be required in the case of parasitoid wasps with long ovipositors that are housed within the body as a spiral (e.g., Eurytomidae) or extended forward in a 'horn' above the thorax (e.g., *Inostemma* species (Platygastridae)).

Three points need to be borne in mind when using the aforementioned technique. First, the insect must be kept covered in saline solution at all times. If it dries out, it cannot be satisfactorily reconstituted. Second, if water rather than saline is used, some structures may expand and become seriously distorted. Finally, unless a fibre-optic system is being used, avoid using an under-stage light source (useful for assisting the examination of some structures) for periods longer than a few minutes, as the specimen will dry out very quickly.

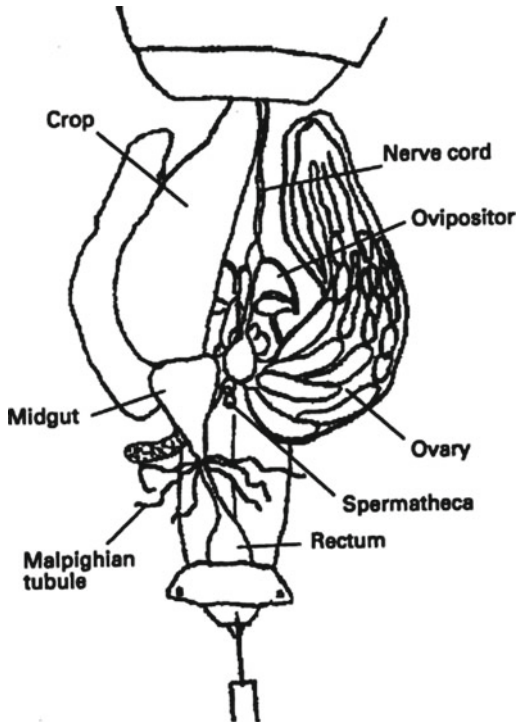


Fig. 2.1 Dissection of the gaster of female *Nasonia vitripennis* (Pteromalidae). The point of a micropin is used to pull away the tip of the gaster and reveal the internal organs

The above technique can be used for small predators and small dipteran parasitoids, but with large insects such as carabid beetles and hover-flies a small, water-filled, wax-bottomed dish should be used instead of a microscope slide and saline droplet. Gilbert (1986) describes a technique for dissecting adult hover-flies (Syrphidae) (Fig. 2.2a) that can also be applied to dipteran parasitoids and predatory beetles and bugs. The insect is placed on its back (dorsal surface) (on a slide or wax-bottomed dish, dry or under saline) and is secured with an entomological pin inserted through the thorax. Using a second entomological pin, a small tear is made in the intersegmental membrane at the junction of the thorax and abdomen. The end of one arm of a fine forceps is then inserted into this hole and the forceps are then used to grip the first abdominal sternite. Then, using a micropin (preferably one having a point that has been slightly bent near its tip), make lateral incisions in the abdomen, following the line of the pleura to the terminalia. Finally, peel back the abdominal sternites to reveal the internal organs (Fig. 2.2b, c). The crop (very large in hover-flies) can be removed in its entirety using forceps, and its contents (pollen and/or nectar) subsequently examined and analysed. The reproductive system can be examined in situ, under saline. Carabid beetles are dissected in a similar fashion, except that the insect is placed on its front (ventral surface). Figure 2.3 shows the gut of a typical carabid beetle.

It is very difficult to interpret the structure of an insect's reproductive system, or that of other organs, if the structure has been fixed and preserved. If a permanent record of a dissection is needed, the insect's organs are best photographed or drawn as soon as possible. Semi-permanent mounts can be made with water-soluble

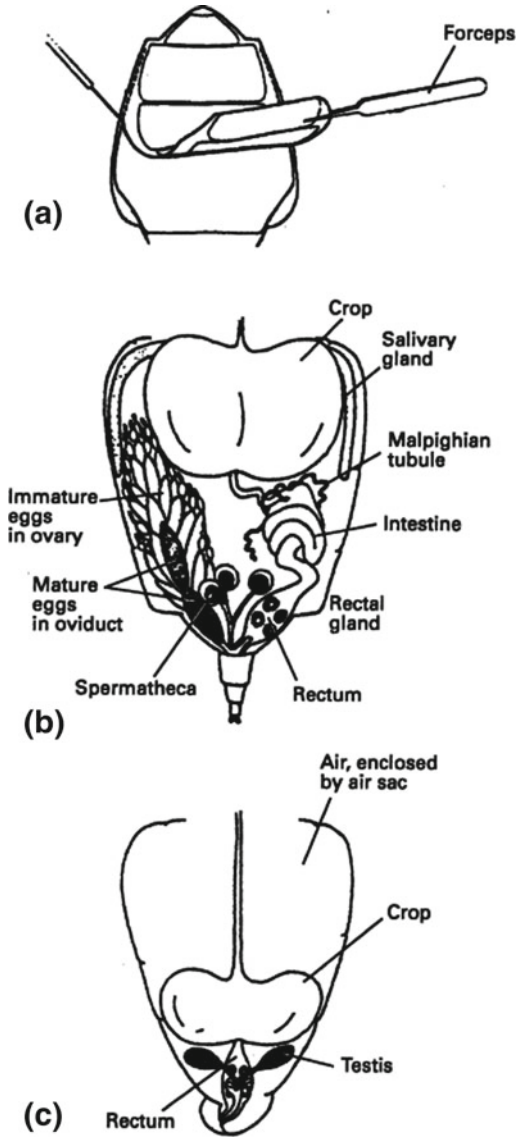


Fig. 2.2 Dissections of hover-fly (Syrphidae) abdomen: **a** dissection procedure; **b** internal anatomy of female, **c** internal anatomy of male. *Source* Gilbert (1986). Reproduced by permission of Cambridge University Press

mountants such as polyvinyl pyrrolidone (Burstone, 1957) or glycerol, but anatomical features are better observed in freshly dissected insects. Anatomical features are enhanced by the use of specialist optics such as phase contrast, interference and dark ground illumination, with a transmission compound microscope.

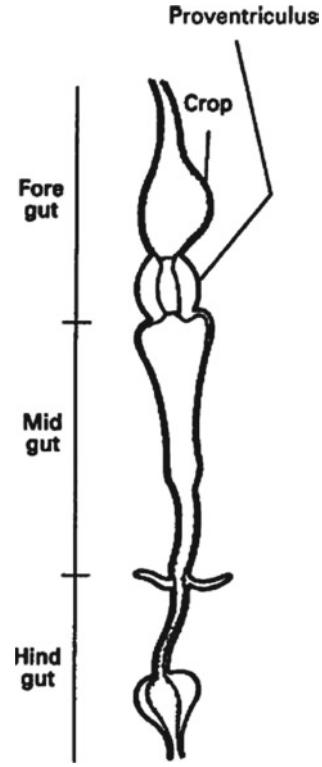


Fig. 2.3 The gut of a typical carabid beetle. *Source* Forsythe (1987). Reproduced by permission of The Richmond Publishing Co. Ltd.

2.2.2.2 Microscopy

There is a limit to the information that can be obtained from dissection. Histological and histochemical techniques will reveal the location of lipids, carbohydrates, nucleic acids and many more specific materials in, for example, the reproductive organs (see also Sect. 2.14). Such techniques have been crucial to our understanding of oögenesis in parasitoids (King et al., 1971; Davies et al., 1986; Reed et al., 2007, Huang et al., 2008 and Bodin et al., 2009). Combined with electron microscopy, they can reveal the detailed structure of secretory tissues, egg oöplasm (e.g., Le Ralec, 1995), and can demonstrate the effects of diet and temperature on structures such as mitochondria and cell membranes. Davies (1974), for example, showed how in *Nasonia vitripennis* the ultrastructure of flight muscle alters with the age of the adult insect and with variations in adult diet.

2.2.3 Ovipositor and Male Genitalia

The ovipositor of female parasitoids may need to be examined in detail in order to understand the mechanics of oviposition, while the secondary genitalia of male dragonflies may need to be examined in order to study sperm competition (Sect. 4.5.2). Light microscopy and scanning electron microscopy (SEM) are usually employed to study these structures. In order to examine whole mounts with light microscopy, clear and stain them following standard protocols, whereas to examine sections, e.g., of ovipositors, embedding, sectioning and staining needs to be carried out; standard protocols (embedding in Spurr's medium and staining, e.g., with Toluidine Blue) were followed, for example, by Austin (1983) and Quicke et al. (1992). Greater detail of external morphology can be seen using SEM (e.g., King & Fordy, 1970; Jervis, 1992; Quicke et al., 1992). Specimens of small Hymenoptera and of Diptera are best prepared for SEM by critical-point drying them (Postek et al., 1980), whereas specimens of larger and more hard-bodied insects require only air drying.

Snodgrass (1935) described the basic structure of both male and female insect genitalia, while Scudder (1971) interpreted the structure of the ovipositor in hymenopterans. For details of ovipositor structure and function in parasitoids, including in some cases the mechanism of egg movement, see Jervis (1992), Field and Austin (1994), Quicke et al. (1994), Le Ralec et al. (1996), Austin and Field (1997), Kozanek and Belcari (1997), Gerling et al. (1998), van Lenteren et al. (1998), Rahman et al. (1998), Le Lannic and Nenon (1999), Vilhelmsen et al. (2001), Heraty and Quicke (2003), Zacaro and Porter (2003), Vilhelmsen (2003), van Lenteren et al. (2007) and Cerkvenic et al. (2017).

Parasitoids, in common with other insects, possess a diversity of sensilla on the ovipositor (Gutierrez, 1970; King & Fordy, 1970; Weseloh, 1972; Hawke et al., 1973; Greany et al., 1977; van Veen, 1981; Jervis, 1992; Kozanek & Belcari, 1997; Cônsoli et al., 1999). The function (i.e., mechanoreception, chemoreception) of the sensilla can be provisionally inferred from their external morphology, but corroboration needs to be obtained by examining them in detail using transmission electron microscopy, by observing female oviposition behaviour, and by carrying out electrophysiological studies. The role of ovipositor sensilla in host acceptance by parasitoids (Sect. 1.5.5) has long been appreciated.

The functional morphology of male genitalia in dipteran and hymenopteran parasitoids has not been extensively studied (Domenichini, 1953; Sanger & King, 1971; Teder, 1998 and Chiappini & Mazzoni, 2000). Recent research in this area has been performed with some egg parasitoids (Paoli et al., 2013; Ramírez-Ahuja et al., 2020). The structure and function of the genitalia of male dragonflies (Waage, 1979, 1984; Artiss, 2001 and Cordoba-Aguilar, 2002), spiders (Eberhard et al, 1998; Rivera-Quiroz et al., 2020) and other insects (Huber et al., 2007) is better understood.

2.3 Female Reproductive Organs

2.3.1 Ovaries

The reproductive organs of hymenopteran (Figs. 2.1, 2.4a, b, d, 2.5) and dipteran (Fig. 2.4c, e) parasitoids comprise a pair of ovaries which themselves comprise several ovarioles in which the eggs (oöcytes) develop. In parasitoid wasps (King & Richards, 1969) and flies (Coe, 1966) the ovarioles are of the polytrophic type. Within each follicle, nurse cells (trophocyte cells: fifteen or more in hymenopteran parasitoids) surround the developing oöcyte, providing it with nutrients (Fig. 2.6a). The oöcyte becomes increasingly prominent as it passes down the ovariole. Each oöcyte, together with its associated trophocyte cells, originates from a single cell. It seems that, in order to develop eggs as rapidly as possible, the protein production machinery of all the trophocyte cells passes materials into the oöcyte. The follicle cells, which may also pass materials from the haemolymph, secrete the chorion (egg membrane). As the oöcyte matures, the trophocyte

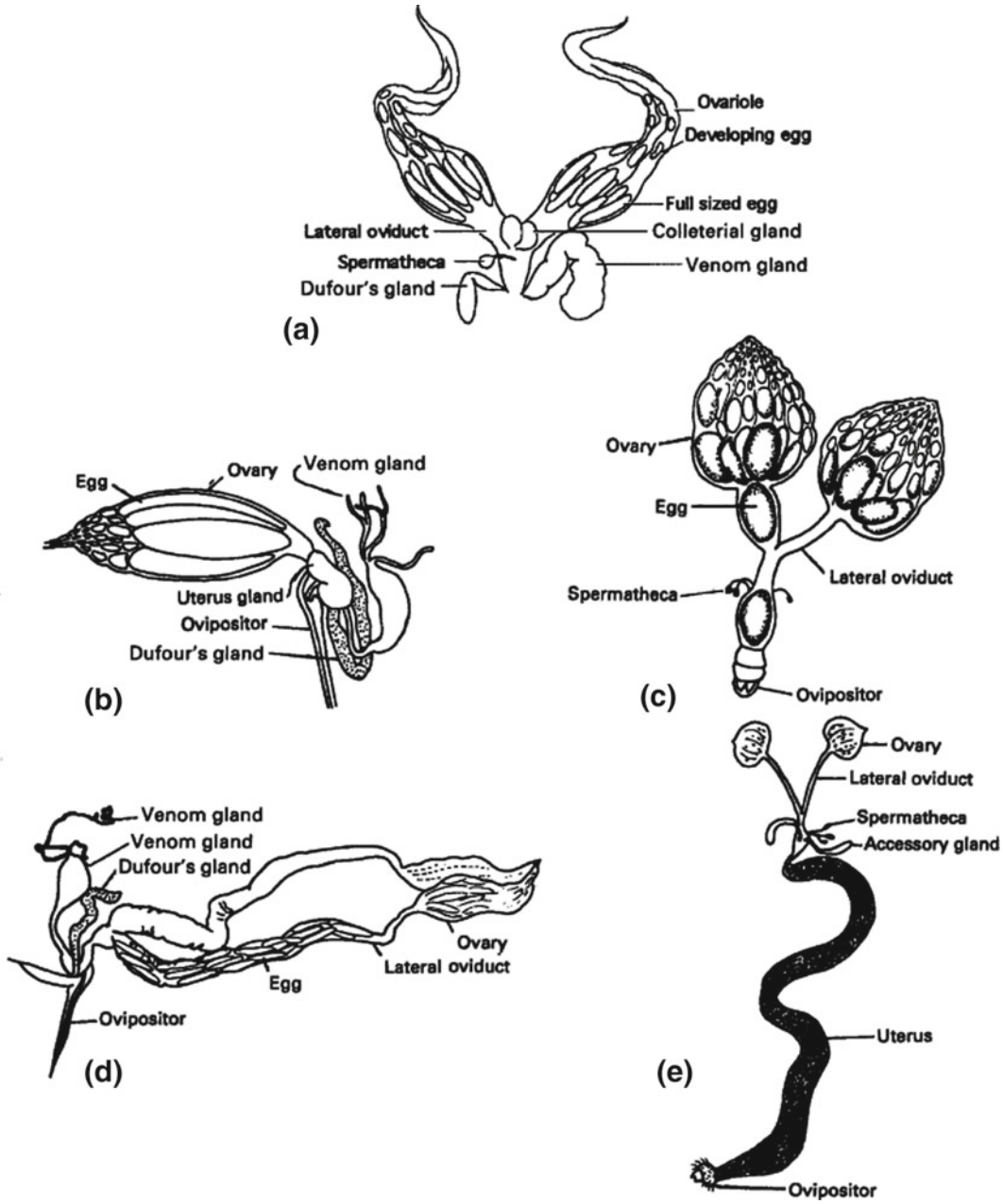


Fig. 2.4 The reproductive systems of some parasitoid wasps and flies: **a** gravid female *Coccophagus atratus* (Aphelinidae) 24 h after emergence (*source* Donaldson & Walter, 1988); **b** *Trachysphyrus albatorius* (Ichneumonidae) (*source* Pampel, 1914, in Price, 1975); **c** *Hyperecteina cinerea* (Tachinidae) (*source* Clausen et al., 1927, in Price, 1975); **d** *Enicospilus americanus* (Ichneumonidae) (*source* Price, 1975); **e** *Leschenaultia exul* (Tachinidae) (*source* Bess, 1936 in Price, 1975). **a** Reproduced by permission of Blackwell Publishing; **b**, **c**, **d** and **e** by permission of Plenum Publishing Corporation

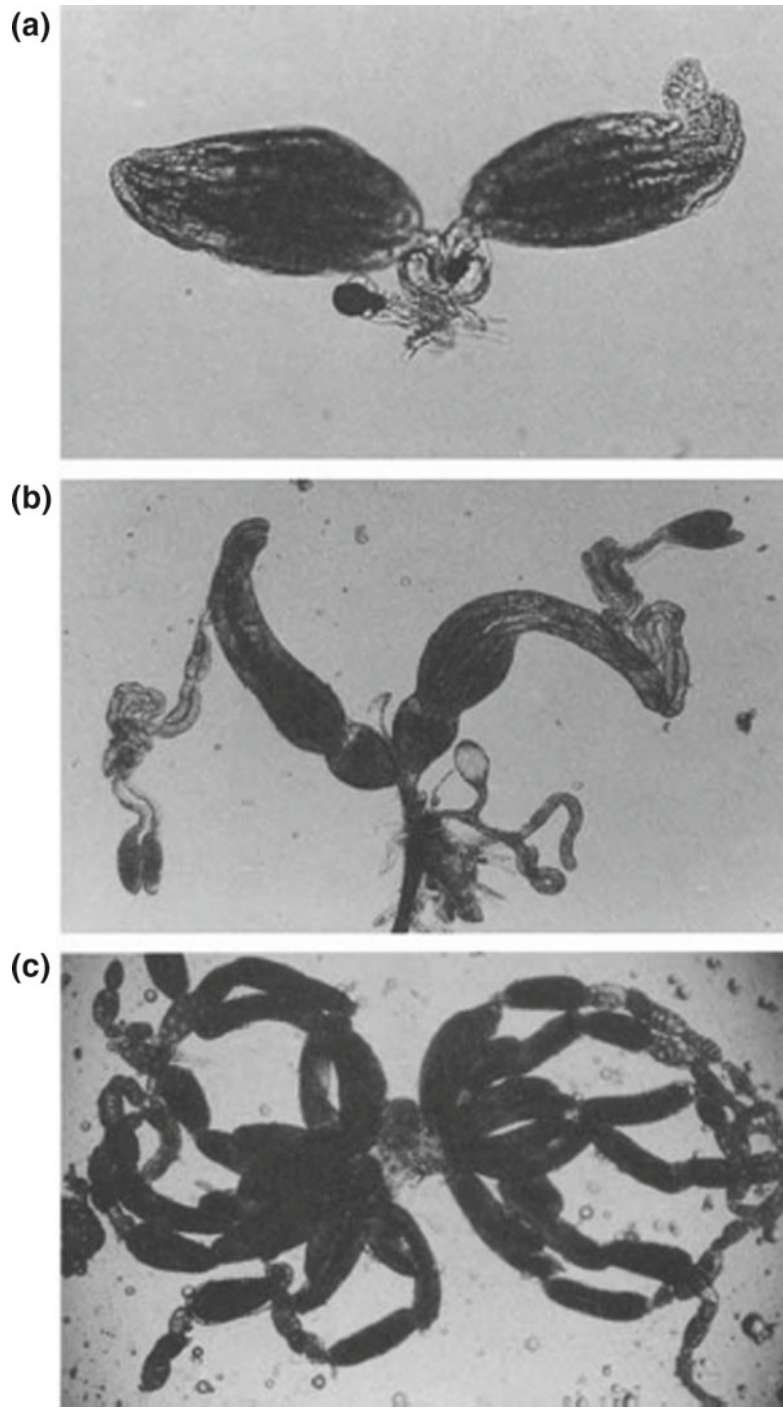


Fig. 2.5 The reproductive systems of some parasitoid wasps: **a** *Gonatocerus* sp. (Mymaridae); **b** *Cotesia* sp. (Braconidae); **c** unidentified Eulophidae

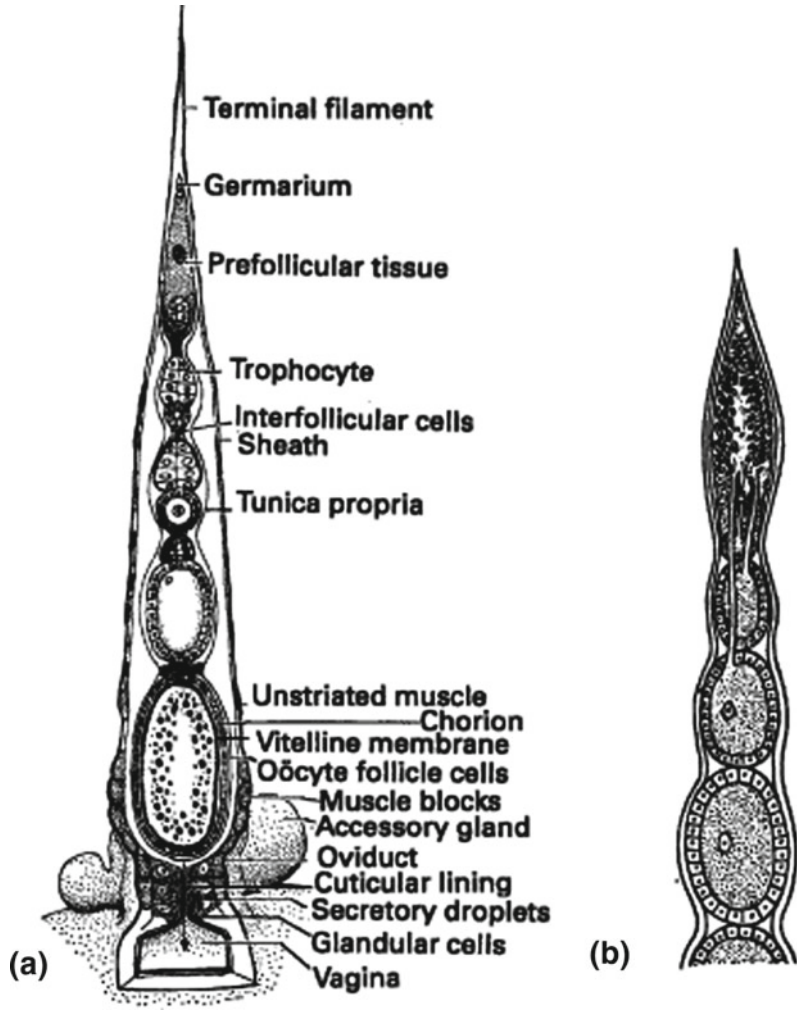


Fig. 2.6 Examples of ovariole structure in natural enemies: **a** polytrophic type, in *Nasonia vitripennis* (Pteromalidae) (*source* King & Ratcliffe, 1969); **b** telotrophic type as found in coccinellid beetles and heteropteran bugs (*source* de Wilde & de Loof, 1973). **a** Reproduced by permission of The Zoological Society of London; **b** by permission of Elsevier Science

cells break down. The follicular epithelium creates a small pore (the micropyle) in the chorion, through which the sperm enters to penetrate the egg membrane and effect fertilisation.

To examine the ovarioles of a dissected insect, remove the ovaries (their attachment to the abdominal wall may need to be severed), place them on a microscope slide in a drop of insect saline, and tease the ovarioles apart with micropins. Then gently place a cover-slip over the ovaries. The number of ovarioles can then be counted and their contents viewed.

In both hymenopteran and dipteran parasitoids, the number of ovarioles per ovary varies both interspecifically (Flanders, 1950; Price, 1975; Jervis & Kidd, 1986; Quicke, 1997; Harvey et al., 2014) and intraspecifically (e.g., van Vianen & van Lenteren, 1986; Harvey et al., 2014; Liu et al., 2014; Ameri et al., 2015). Ovarian structure often differs markedly between koinobiont and idiobiont parasitoids. For instance, koinobionts are often much more highly fecund than idiobionts, and this is reflected in the number of ovarioles per ovary, which is often far greater than in idiobionts (Flanders, 1950; Price, 1972; Jervis et al., 2001, 2008; Harvey, 2008). Many chalcidoid wasps have an average of three ovarioles per ovary (*Encarsia formosa* has an average of eight to ten, depending on the population studied), whereas in ichneumonoid wasps the range of interspecific variation is much wider (Iwata, 1959, 1960,

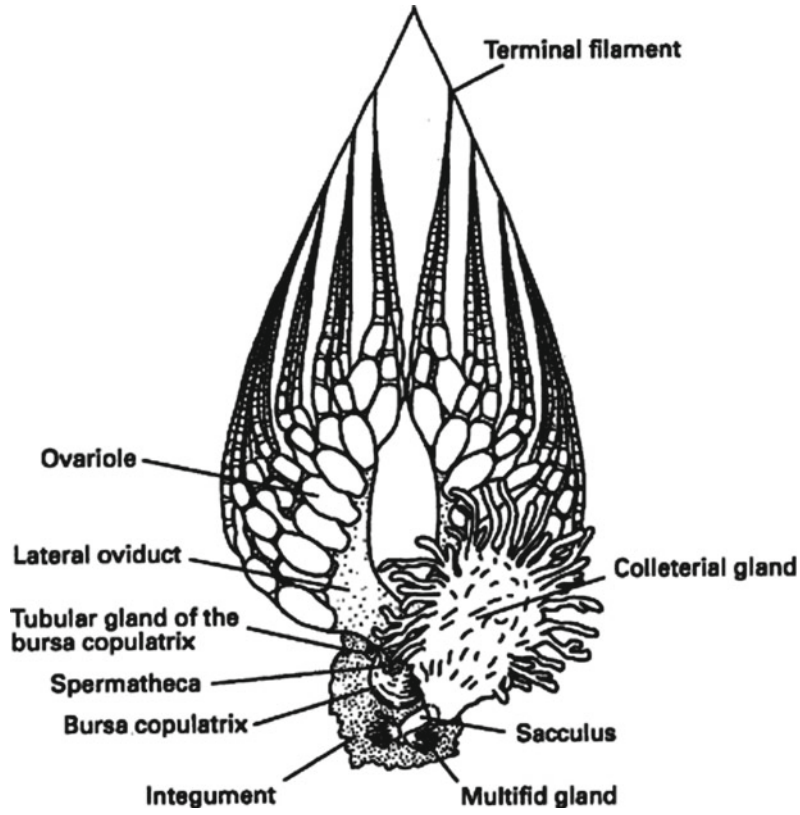


Fig. 2.7 Schematic representation of reproductive system in female *Chrysopa septempuncata* (Neuroptera), dorsal aspect. *Source* Principi (1949). Reproduced by permission of W. Junk, Publishers

1962; Cole, 1967; Quicke, 1997). In some species of Ichneumonidae ovariole number alters according to whether the females are of the first or the second field generation, female body size being taken into account, i.e., there is a seasonal dimorphism (Cole, 1967). In predatory coccinellids, as in parasitoids, there is both intra- and interspecific variability in ovariole number (Iperti, 1966; Stewart et al., 1991). Welch (1993) reviews ovariole number in Staphylinidae.

Predator ovaries fall into several categories. Those of chrysopid lacewings and carabid and gyrinid beetles have polytrophic ovarioles (e.g., Fig. 2.7), but coccinellid beetles and predatory heteropteran bugs have telotrophic ovarioles (Fig. 2.6b). In the latter, the trophocyte cells, instead of accompanying the oöcyte as it moves down the ovariole, remain in the swollen distal end and remain attached to the egg by a lengthening cytoplasmic strand that conveys the nutrients. Telotrophic ovarioles are therefore short, but they are often numerous.

A measure of female reproductive potential can be obtained by counting the total number of oöcytes (mature and immature) within the ovaries and oviducts (Sect. 2.7.1). It is a fairly simple procedure to count the number of mature eggs in species that possess enlarged lateral oviducts in which the eggs accumulate (Sect. 2.3.2), but care is needed in the case of species that store (albeit for a brief period) some or all of their eggs within the basal part of the ovariole. With practice, it is possible to recognise mature eggs by their slightly opaque appearance resulting from the presence of yolk within (i.e., in anhydropic species, Sect. 2.3.4). Immature oöcytes, particularly the smaller ones, are more difficult to count. A stain such as acetocarmine can be used to reveal them more clearly: the stain is taken up by these oöcytes, because they lack a chorion (in mature oöcytes, only the surrounding follicle becomes stained; the follicle is eventually lost prior to the mature egg entering the oviduct).

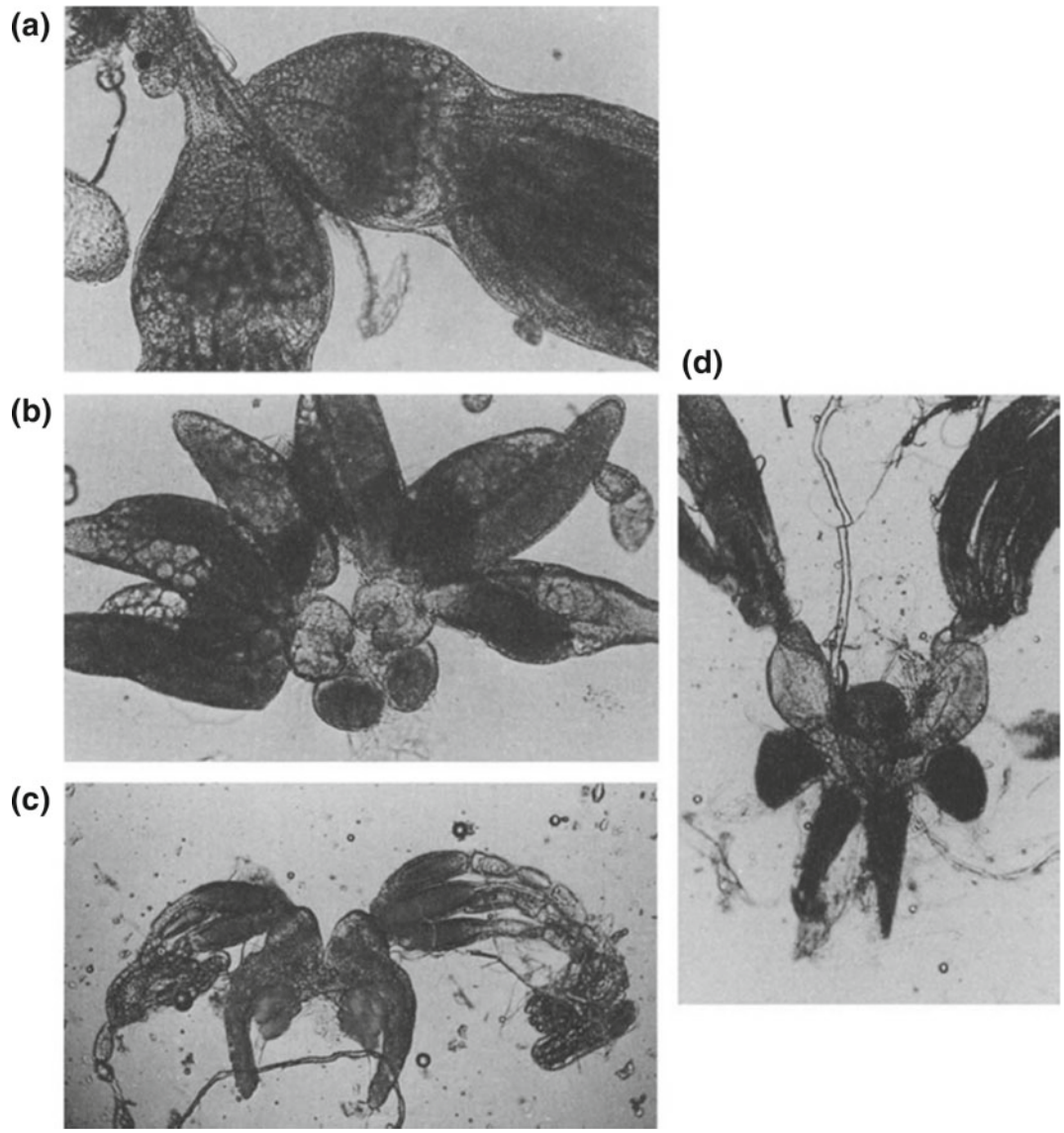


Fig. 2.8 The calyx region of lateral oviduct in: **a** *Cotesia* sp. (Braconidae); **b** *Aprostocetus* sp. (Eulophidae) (also showing one pair of colleterial glands); **c** *Torymus* sp. (Torymidae) (also showing two pairs of colleterial glands); **d** *Macroneura vesicularis* (Eupelmidae) (showing calyx lobes, i.e., the very long structures, and two pairs of colleterial glands)

2.3.2 Oviducts

The ovarioles empty into the lateral oviducts (Figs. 2.4, 2.5, 2.8). In most Hymenoptera, each lateral oviduct includes an obvious glandular region, the calyx (Fig. 2.8), which secretes materials onto the egg as it is laid (Rotheram, 1973a, b). In some Braconidae and Ichneumonidae, the calyx is the source of polydnaviruses (baculoviruses of the family Polydnaviridae) (Stoltz & Vinson, 1979; Stoltz, 1981; Strand et al., 1988; Fleming, 1992; Bézier et al., 2009; Herniou et al., 2013). The latter, which replicate in the cells of the calyx, play a role in preventing encapsulation of the parasitoid egg (Sect. 2.10.2) and in modifying the host's growth, development, morphology and behaviour (Vinson

& Iwantsch, 1980a; Stoltz, 1986; Strand et al., 1988; Beckage, 1998a, b; Webb, 1998; Strand & Burke, 2014; Ye et al., 2018). Chelliah and Jones (1990) raised an antibody against the extracted polydnaviral proteins of *Chelonus* sp. and then used it to reveal the location of such proteins in the wasp's reproductive system.

In some synovigenic parasitoid wasps the lateral oviducts can accommodate a small number of eggs, e.g., 9–12 per oviduct in *Coccophagus atratus* (Donaldson & Walter, 1988) (anhydropy, Sect. 2.3.4). In others the oviducts are greatly elongated, to form distinctive 'uteri', and can accommodate very large numbers of small eggs (Figs. 2.4d and 2.5b) (hydropy, Sect. 2.3.4).

The lateral oviducts join to form the common oviduct, a largely muscular structure that in turn becomes confluent with the vagina and (in wasps) the ovipositor stylets. In some tachinid parasitoids, egg storage (and incubation) occurs in the common oviduct, e.g., *Cyzenis albicans* (Hassell, 1968). In wasps, forward-pointing spines in the vagina push the egg into the ovipositor at or before oviposition (Austin & Browning, 1981). As it passes down the ovipositor, the egg is squeezed to a small diameter, a process that has been shown to trigger embryonic development (Went & Krause, 1973). Embryonic development of haploid (male) eggs of the ichneumonid parasitoid *Pimpla turionellae* can also be triggered by experimental injection, not involving egg deformation, of calcium ionophore A23187 (Wolf & Wolf, 1988). The chorion of the hymenopteran egg is remarkably flexible, so experiments on the initiation of embryogenesis can be carried out on mature eggs that have been removed from the ovarioles or lateral oviducts of a wasp. The eggs can be manipulated in various ways on a microscope slide, in saline solution, to show, for example, what degree of compression is required to trigger embryogenesis. In the tachinid *Cyzenis albicans* eggs, when laid, contain a fully formed first-instar larva (Hassell, 1968).

2.3.3 Shape, Size and Number of Eggs

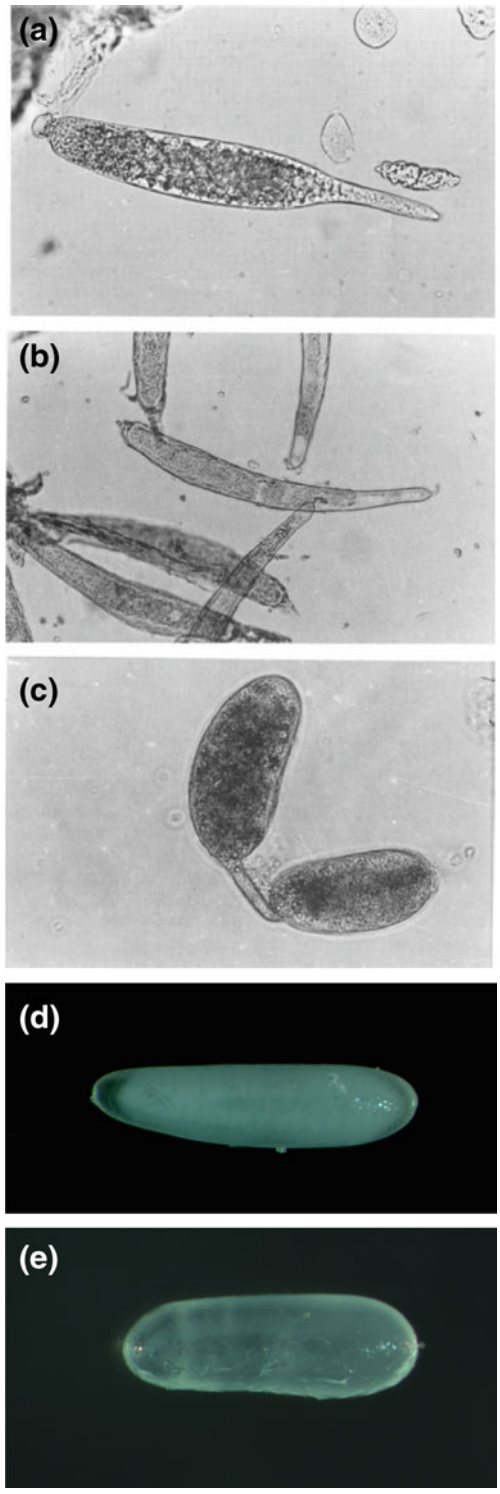
The shape of eggs in parasitoid wasps and flies varies considerably between groups (Iwata, 1959, 1960, 1962; Hagen, 1964; Quicke, 1997). Egg types found among parasitoid wasps include those with a simple ovoid shape, those that are greatly elongated (Fig. 2.9a, b), those with a distinctive stalk at the micropyle end, and those with a double-bodied appearance (Fig. 2.9c). For a review of the range of egg types found among parasitoids, see Hagen (1964) and Quicke (1997).

Some eggs (hydropic-type eggs, Sect. 2.3.4) characteristically increase greatly in size following deposition in the host's haemocoel. Among Braconidae, for example, eggs of Euphorinae expand in volume a thousand times (Ogloblin, 1924; Jackson, 1928), and those of *Praon palitans* (Aphidiinae) over six hundred times (Schlinger & Hall, 1960).

Within a parasitoid wasp species, the number and the size of mature oöcytes in the ovaries are, in general, positively correlated with the size of the female (e.g., O'Neill & Skinner, 1990; Rosenheim & Rosen, 1992; Visser, 1994; but see Fitt, 1990). This observation has important implications for foraging models, since larger females may, theoretically, obtain larger fitness returns per host and also, compared with smaller females, they can utilise a series of hosts in more rapid succession (Skinner, 1985; O'Neill & Skinner, 1990).

The number of mature oöcytes in the ovaries is a function of the number of ovarioles, which is also correlated with body size within a species (e.g., Branquart & Hemptinne, 2000). Data on oöcyte number, oöcyte size and ovariole number have been gathered for a limited number of species. In spiders, it was shown that the amount of metabolic energy invested per egg is species specific and strongly influences egg size (Anderson, 1990).

In the damselfly *Coenagrion puella*, the carabid beetle *Brachinus lateralis*, and the hoverfly *Episyrphus balteatus*, egg size is not



◀ **Fig. 2.9** Eggs of parasitoid Hymenoptera. Eggs dissected out of the reproductive systems of parasitoid Hymenoptera: **a** unidentified Mymaridae; **b** *Cotesia* sp. (Braconidae); **c** unidentified Encyrtidae. Stereomicroscopic images of laid eggs: **d** *Habrobracon hebetor* (Braconidae); **e** *Goniozus nephantidis* (Bethylidae) (Photographs **d** and **e**: K.S. Shameer)

correlated with female size (Juliano, 1985; Banks & Thompson, 1987a; Branquart & Hemptinne, 2000), but it is positively correlated with body size across species of Gerridae and predatory Coccinellidae (Kaitala, 1991; Dixon, 2000).

The size of a female's eggs may alter during her lifetime. Giron and Casas (2003b) demonstrated that *Eupelmus vuilletti* reduces egg provisioning with age: with increasing age, there is a marked decrease in reproductive investment with respect to egg size, and sugar, protein, lipid and energy content. Egg size was a good predictor of offspring fitness, measured as survival of neonate larvae. Wallin et al. (1992) showed that in carabid beetles egg size decreases with increasing oviposition rate.

Between parasitoid species, ovariole number is a good predictor of fecundity, as Price (1975) has shown for Ichneumonidae and Tachinidae (Fig. 2.10). It remains to be tested whether or not a correlation exists between body size and ovariole number, on a broad, between-species basis.

Blackburn (1991a) and Jervis et al. (2003) showed, through comparative analyses, that among parasitoid wasps there is not a positive relationship between adult size and lifetime fecundity (fecundity is defined in Sect. 2.7.1), although Blackburn (1991a) detected such a relationship when he controlled for egg size. When adult size is controlled for, species with a high fecundity (the maximum number of eggs reported to have been laid by an individual of a species) tend to have smaller eggs, indicating a trade-off between fecundity and egg size (small eggs require less of a material investment) (further discussed in Blackburn, 1991a). Mayhew and Blackburn (1999) showed, also through a comparative analysis, that koinbionts produce smaller eggs than do idiobionts.

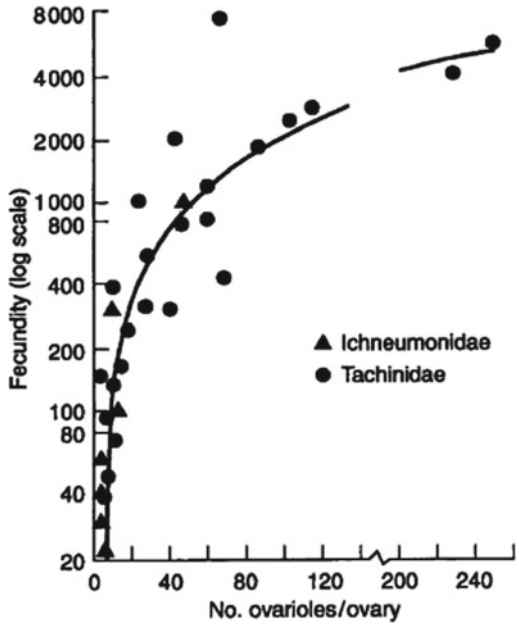


Fig. 2.10 The relationship between fecundity (note log scale) and the number of ovarioles per ovary in species in the Ichneumonidae and in the Tachinidae. Data points represent means for individual species. *Source* Price (1975). Reproduced by permission of Plenum Publishing Corporation

The interspecific relationships among predatory Syrphidae and among Coccinellidae with respect to ovariole number, mature oöcyte number, oöcyte size and female body size, and their biological significance, are discussed by Gilbert (1990) and Dixon and Guo (1993). Note that in predatory coccinellids large species produce proportionately smaller eggs, relative to their body size, than smaller ones. For a discussion of the adaptive significance of the egg size–body size relationship in the Coccinellidae, see Dixon (2000).

2.3.4 'Ovigeny' and Related Traits

2.3.4.1 Ovigeny Index

Among insects, even among members of the same order, there may be considerable variation in the degree to which the female's lifetime potential egg complement is mature when she emerges into the environment following pupal development. For example, the orders Lepidoptera and Hymenoptera each include, at one extreme, species that emerge with a fully developed lifetime egg complement and, at the other extreme, species that emerge with only immature oöcytes (Flanders, 1950; Dunlap-Pianka et al., 1977; Jervis et al., 2001). There are even intraspecific, intra-population genetic variations in this trait (Wajnberg et al., 2012). The 'ovigeny index', which is expressed as the proportion of the initial mature egg load that make up the lifetime potential fecundity (Sect. 2.7.1), was devised by Jervis et al. (2001) to quantify variation in the degree of egg development shown by insects both interspecifically and intraspecifically. Ovigeny index = 1 ('strict pro-ovigeny' sensu Jervis et al., 2001) indicates that all the female's oöcytes are mature upon emergence, whereas ovigeny index = 0 ('extreme synovigeny') denotes emergence with no mature oöcytes. A continuum of ovigeny index values exists among parasitoid wasps, ranging from strict pro-ovigeny, through weak then strong synovigeny, to extreme synovigeny (Jervis et al., 2001); the same probably applies to parasitoid Diptera and also insect predators as a whole.

The numerator in the calculation of ovigeny index—initial egg load (the number of mature, i.e., fully chorionated [layable] eggs in newly emerged females)—is in many species easily measured through dissection. Lifetime potential fecundity, the denominator in the calculation of ovigeny index, is measured by adding the number of immature oöcytes (also measured through dissection) to the initial egg load. Alternatively, it can be approximated by measuring the average lifetime realised fecundity (Sect. 2.7.1) achieved under conditions of high host abundance (hosts supplied ad libitum) and high food availability/quality.

The ovigeny index can be used as a simple measure of the allocation of resources to reproduction at the start of adult life (Sect. 2.13.2), and thus to seek some of the classic trade-offs predicted by general life-history theory (Bell & Koufopanou, 1986; Smith, 1991; Stearns, 1992; Roff, 2002). For example, in parasitoid wasps, ovigeny index and life-span are negatively

correlated both within species (Jervis et al., 2001, using data in Ellers & van Alphen, 1997) and across species (Jervis et al., 2001, 2003), suggesting that there is a cost, to life-span, of concentrating reproductive effort into early adult life (Jervis et al., 2001). At least within species, the negative correlation is attributable to the differential allocation of capital resources between initial eggs on the one hand, and fat body reserves (which contribute to maintenance metabolism) on the other (Ellers & van Alphen, 1997) (Sect. 2.13.2). Ovigeny index has also been used to explore the body size-related trade-off between current and future reproduction (Ellers & Jervis, 2003).

Other life-history variables found to be correlates of ovigeny index are: egg resorption capability (associated with a low index), egg type (hydropy is associated with a high index, anhydropy with a low index), and body size (negatively correlated with ovigeny index, both between and within species) (Jervis et al., 2001, 2003; Ellers & Jervis, 2003). Host-feeding species tend to have a low index, as do idiobionts (Jervis et al., 2001). Ovigeny index is hypothesised to be correlated with the degree of resource carry-over (i.e., from pupa to adult) (Sect. 2.13.2): an index of 1 indicates that the materials used for lifetime reproduction derive entirely from larval resources, whereas indices of <1 indicate that the materials used for lifetime reproduction derive only partly from carried-over resources, the females relying upon external nutrient inputs to mature their remaining oöcytes). This difference in life-history strategy closely parallels the concept of 'capital' *versus* 'income' breeding (Drent & Daan, 1980; Boggs, 1992, 1997a). The ovigeny index can also be affected by abiotic factors such as temperature. For example, Moiroux et al. (2018) found that ovigeny index in the synovigenic parasitoid *Aphidius ervi* increased when immature stages or adults were exposed to higher temperatures. If more broadly applicable, these results could have implications on parasitoid reproductive behaviour and demographics in the field, especially under conditions experienced during climatic extremes (Harvey et al., 2020; Ma et al., 2021).

For details of the criteria used in deciding whether a species is strictly pro-ovigenic or synovigenic, see Jervis et al. (2001). Note that some species categorised by authors as pro-ovigenic are, in reality, weakly synovigenic (Mills & Kuhlmann, 2000; Jervis et al., 2001).

2.3.4.2 Autogeny/Anautogeny in Synovigenic Insects

Presumably due to there being insufficient resource carry-over from the larval stage, some synovigenic species can mature some eggs without first feeding (i.e., are autogenous), whereas others must feed (i.e., are anautogenous). It is likely that the vast majority of koinobiont endoparasitoids that produce hydropic eggs are autogenous (Jervis & Kidd, 1986; Harvey, 2005; Pennacchio & Strand, 2006; Jervis et al., 2008). Hover-fly (Syrphidae) species are synovigenic-autogenous (Gilbert, 1991). The tachinid *Cyzenis albicans* is synovigenic-autogenous (Hassell, 1968). Predatory coccinellids are synovigenic-anautogenous. The green lacewing *Chrysoperla carnea* is anautogenous when reared only on prey, but is autogenous when given a non-prey food, together with prey, during larval life (McEwen et al., 1996). In anautogenous host-feeding species, the females must consume host haemolymph in order to mature eggs (Jervis & Kidd, 1986).

2.3.4.3 Hydropy and Anhydropy

Flanders (1942) distinguished between two types of egg in parasitoid wasps, hydropic and anhydropic, based on the function of the chorion. Hydropic eggs, which are restricted to endoparasitoid species, usually swell to a considerable degree within hours or a few days of being deposited within the host's haemolymph (Schlinger & Hall, 1960). Compared with the mature ovarian eggs, the swollen eggs in euphorine Braconidae are 1000 times larger in terms of volume. The swelling occurs as a result of the uptake, via the thin, permeable chorion, of components of the host's haemolymph (Ferkovich & Dillard, 1987). In hydropic egg-producing parasitoids, the permeable chorion is connected physically to the embryo via an extra-

embryonic membrane, which absorbs nutrients from host haemolymph during embryogenesis (Grbić & Strand, 1998). Anhydropic eggs, which occur among ectoparasitoid as well as endoparasitoid species, have a relatively thick, rigid, impermeable chorion, and any apparent swelling they undergo is slight and mostly the result of the embryo having developed into the first-instar larva.

Hydropic eggs contain little yolk, which is mainly comprised of lipids (Le Ralec, 1995). Their oöplasm contains numerous ribosomes and mitochondria, both organelles apparently being derived from the female's trophocytes, via the nutritive pore (King et al., 1971; Le Ralec, 1995). Proteins, rather than being acquired from the host's haemolymph, are synthesised *de novo* within the oöplasm, from amino acids which have been obtained from the host (Ferkovich & Dillard, 1987). The major contribution by the mother to its progeny is thus a protein synthesis apparatus to enable complete embryonic development (Le Ralec, 1995). Anhydropic eggs, by contrast, contain much yolk. Their oöplasm contains numerous lipoid bodies. Proteins, mainly composed of vittelin, are also present, but their character varies among species. In species whose females consume host haemolymph ('host-feed', Sect. 1.8), the protein bodies are typical of insects generally (King & Richards, 1969; Kunkel & Nordin, 1985; Le Ralec, 1995) but in species that do not host-feed they appear to be atypical, although their biochemical composition has yet to be clarified (Le Ralec, 1995). In anhydropic egg-producing species, the mother contributes to its progeny sufficient sources of both energy-rich (lipid) and nitrogen-rich (protein) materials to enable embryonic development to be completed. Harvey (2008) compared reproduction and development in two species of closely related secondary (hyper)parasitoids in the ichneumonid subfamily Cryptinae, *Lysibia nana* and *Gelis agilis*, both of which attack cocoons of *Cotesia glomerata*. Each species produces anhydropic eggs and both have ovigeny indices of 0. However, whereas adult female *G. agilis* obligatorily host-feeds before producing eggs, *L. nana* does not. This reveals that phylogeny plays some role in explaining the expression of some reproductive traits but not others.

It is reasonable to conclude from the above that the greatest degree of parental (female) investment per egg is made by anhydropic egg-producing species. Indeed, Godfray (1994) and Mayhew and Blackburn (1999) assumed the selection pressures for divergence in egg size among parasitoids to be linked to the selection pressures for divergence in egg type (hydropy/anhydropy), with the result that small egg size is associated with hydropic egg production, and large egg size associated with anhydropic egg production. Jervis et al. (2001, 2003, 2008) therefore took hydropy and anhydropy to be proxy measures of such investment when seeking a link between egg type and the timing of egg production (ovigeny index). In a comparative analysis of over 60 parasitoid wasp species, hydropic egg-producing species were shown to have, on average, a significantly higher ovigeny index than anhydropic species. Given that Jervis et al. (2003) have shown ovigeny index to equate with initial egg load, the aforementioned result accords well with the trade-off, between egg number and egg size across species, predicted for animals generally by life-history theory (Smith & Fretwell, 1974), and established empirically for parasitic (mainly parasitoid) wasps by Berrigan (1991). Therefore, the hydropy/anhydropy distinction would seem to be a valid comparative measure of parental investment per egg. A more convincing case in support of this assumption could be made if egg type and egg volume were shown to be positively correlated. An alternative approach would be to show that hydropy and anhydropy are linked to cross-species variation in body size. The rationale behind the existence of such a relationship is that in parasitoid wasps, egg volume and body size are positively correlated, irrespective of the method by which volume is calculated (Berrigan, 1991; Blackburn, 1991a). Ideally, future research into interspecific patterns of maternal egg provisioning should involve measuring allocation per egg in terms of total energy and of the amounts of key nutrients, using the techniques applied by Giron and Casas (2003b) to *Eupelmus vuilletti*.

2.3.4.4 Egg Resorption

In synovigenic-anhydropic parasitoids, oöcytes, when they become mature, are not immediately discharged into the lateral oviduct. Usually a maximum of only a few (three in *Encarsia formosa;* van Lenteren et al., 1987) mature eggs can be stored per ovariole at any moment in time. These eggs, however, can be retained for only a brief period of time, as they have limited storage life, and space has to be made for other mature oöcytes to enter the lateral oviduct. If a female is deprived of hosts for a sufficiently long period (i.e., hosts are absent or are otherwise very scarce), she does not jettison such eggs but begins resorbing them, commencing with the oldest (see below) (see also Stokkebo & Hardy, 2000). In *Nasonia vitripennis* only the pycnotic residue of the follicle cell nuclei remains after resorption (King & Richards, 1968), although in a few species females may deposit partially resorbed eggs (Flanders, 1950). In some cases, even developing oöcytes may be resorbed (reviewed by Jervis & Kidd, 1986, and van Lenteren et al., 1987). By resorbing eggs, the female can use the energy and materials obtained from the eggs to maintain herself and to sustain ovigenesis until hosts are again available. Through egg resorption, eggs are returned to the body of the wasps with only a partial loss of energy and materials, instead of the total loss that would occur if the eggs were jettisoned. In the mymarid parasitoid *Anaphes nitens*, the rate of egg resorption is higher in starved wasps than wasps fed with honey (Carbone et al., 2008). This suggests that the presence of carbohydrates (sugars) inhibits the need for parasitoids to resorb nutrients in their eggs, and suggests that egg resorption is a last-resort survival tactic (Jervis & Kidd, 1986). Egg resorption can be a form of egg limitation in synovigenic parasitoids, since whilst a female is in the process of resorbing eggs, she may be temporarily incapable of ovipositing even if hosts become available (Jervis & Kidd, 1986, 1999; Heimpel & Rosenheim, 1998).

Eggs that are undergoing resorption can be detected at the proximal ends of the ovarioles by their unusual shape (and sometimes colour in hemipteran bugs) compared with unaffected eggs (Fig. 2.11a, b). Because of the partial removal of the chorion, eggs that have recently begun to be resorbed may, unlike unaffected eggs, increase in size when dissected out in water, and will certainly take up stains such as acetocarmine or trypan blue more readily (King & Richards, 1968).

As they are being resorbed, eggs shrink and finally disappear, leaving remnants of the exochorion. The latter are probably voided through the egg canal at the next oviposition, although in some Encyrtidae part of the chorion (the aeroscopic plate) remains in the ovariole or is voided into the haemocoel (Flanders, 1942).

The time of onset of resorption in host-deprived wasps varies, depending on the availability of food. A female *Nasonia vitripennis* or *Goniozus nephantidis* that is starved will begin resorbing eggs earlier than a female that is given honey (Edwards, 1954; Stokkebo & Hardy, 2000). Heimpel et al. (1997a) recorded egg resorption in starved *Aphytis melinus* but not in honey-fed ones over the 36-h experimental period. In host-deprived, honey-fed females of *Nasonia vitripennis* oöcyte development continues, albeit slowly. Among starved female *Phanerotoma franklini*, some females apparently did not live long enough to resorb eggs, whereas sugar-fed females monitored to natural death began to resorb eggs after around 30 days, and by 40 days had resorbed all of their eggs (Sisterton & Averill, 2002).

The rate of egg resorption can be measured using the chemical colchicine, which stops cell division by interfering with microtubule formation, and therefore halts production of further mature eggs. Rates measured for parasitoids vary from one to several days (Edwards, 1954; Bartlett, 1964; Benson, 1973; Anunciada & Voegelé, 1982; van Lenteren et al., 1987). In completely starved *Nasonia vitripennis*, when the terminal oöcyte of one ovariole has begun to be resorbed, it is followed by those in other ovarioles. With continued starvation, the penultimate oöcyte will also start being resorbed, first in one ovariole and then in the others, and so on (King & Richards, 1968).

If a female parasitoid is deprived of hosts for a long enough period for resorption to commence, the number of mature oöcytes in the ovaries (egg

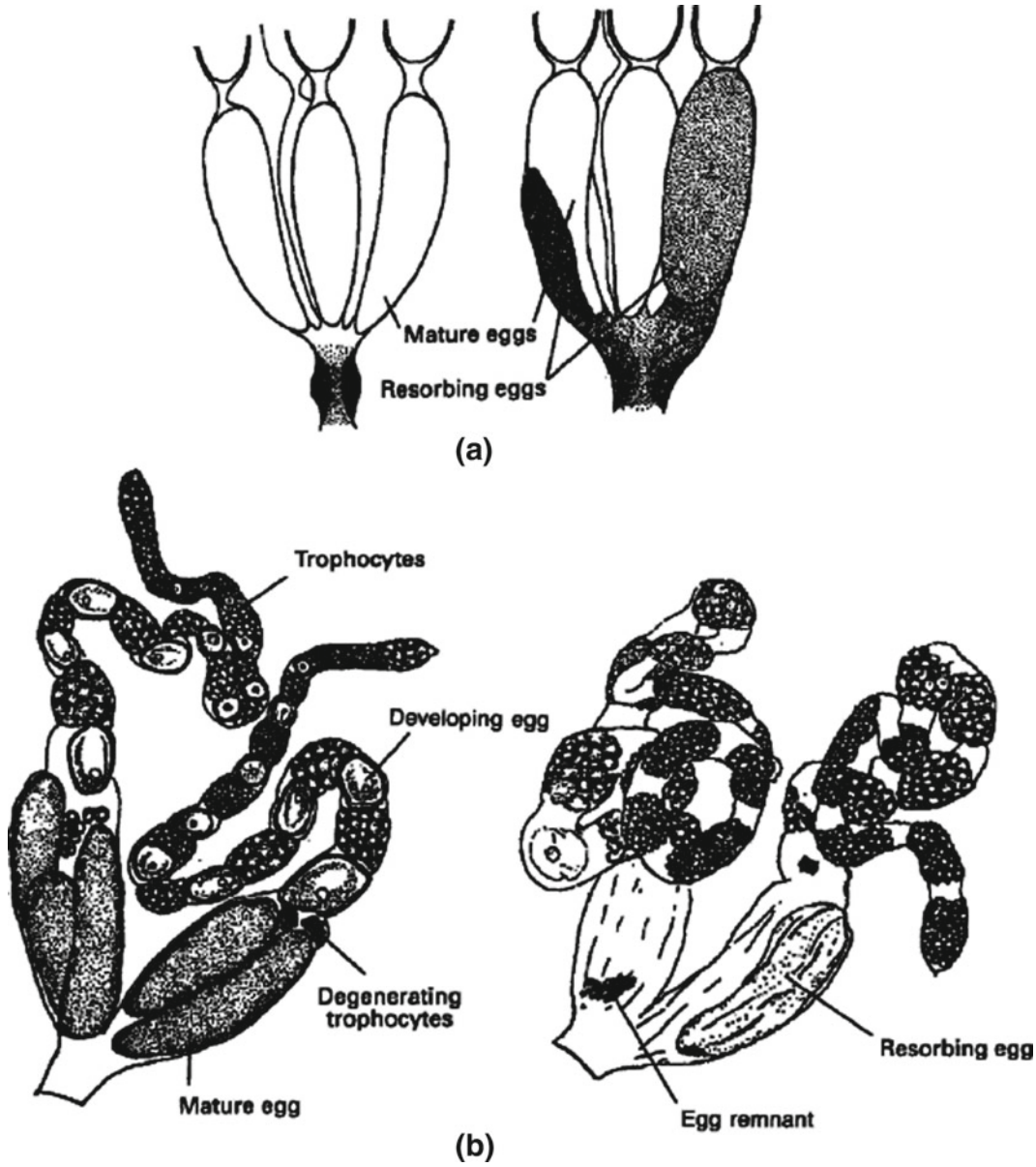


Fig. 2.11 Egg resorption in synovigenic-anhydropic parasitoid wasps: **a** *Nasonia vitripennis* (Pteromalidae) (*source* King & Richards, 1968); **b** *Habrobracon hebetor* (Braconidae). (*source* Grosch, 1950) [In both cases, the ovarioles of a non-resorbing female are shown on left, and those of a resorbing female are shown on right]. **a** Reproduced by permission of The Zoological Society of London; **b** by permission of The Marine Biological Society, Woods Hole, Massachusetts

load), will depend on both: (a) the rate of oögenesis (which will be much lower in starved females than in females that have access to non-host foods, Sect. 2.7.3) and (b) the rate of resorption (King, 1963; van Lenteren et al., 1987).

2.3.5 Egg Limitation

As discussed in Chaps. 1 (Sect. 1.16.2) and 7, the degree to which a parasitoid is egg limited is an important consideration when studying parasitoid

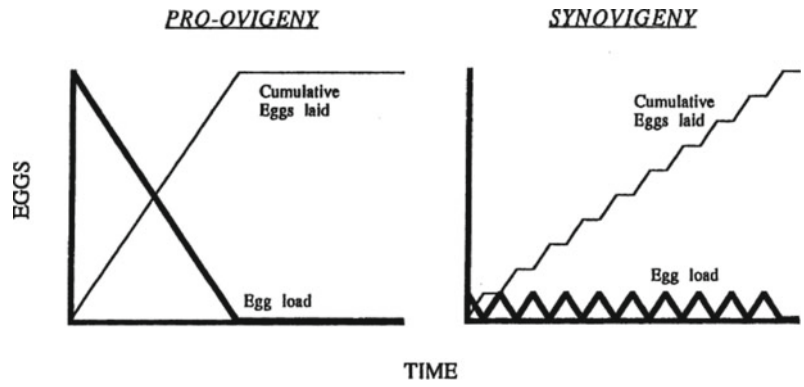


Fig. 2.12 The changes in egg load and the cumulative number of eggs laid by a strictly pro-ovigenic and a strongly synovigenic species in relation to successive oviposition events. From Heimpel and Rosenheim (1998). Reproduced by kind permission of Elsevier Science

foraging behaviour, from the standpoints of fitness gain and searching efficiency. The size of the parasitoid's mature egg load determines the number of eggs the female can lay at a given moment in time (Heimpel & Rosenheim, 1995) (Fig. 2.12). What, then, sets the upper limit to egg load: is it the rate of ovigenesis or the storage capacity?

If, in a species that is not currently resorbing eggs, not all the ovarioles are found to contain a mature egg at any instant in time when ovigenesis is at its maximum, i.e., there is asynchrony among ovarioles, then the ceiling to egg load is set by the rate of ovigenesis, not by the storage capacity. On the other hand, if at any time all the ovarioles contain a full-sized egg and the lateral oviducts are also full of eggs, then the ceiling is likely to be set by storage capacity (in which case one must ask: does ovigenesis cease when the maximum storage capacity is reached?). *Coccophagus atratus* apparently belongs to the second category. If females of this species are withheld from hosts but fed on honey following eclosion and are dissected after varying periods, the egg load is found to increase during the first 24 h of adult life and thereafter remain constant (Fig. 2.13). Since in this species there is no evidence for egg resorption, egg numbers are probably limited by the storage capacity of the ovarioles/lateral oviducts, with ovigenesis ceasing when there is no room for further eggs (Donaldson & Walter, 1988). In the solitary koinobiont endoparasitoid *Venturia canescens*, egg storage capacity in the oviducts is reached in host-deprived females around five days after eclosion (Harvey et al., 2001). At this point oögenesis ceases until females parasitise multiple hosts, when it resumes. By contrast, in some idiobiont parasitoids, egg limitation is taken to the extreme. For example, the cryptine facultative hyperparasitoid *Gelis agilis* has only two ovarioles per ovary and can store no more than two anhydropic eggs in them at a given time. As a result, daily and lifetime fecundity under

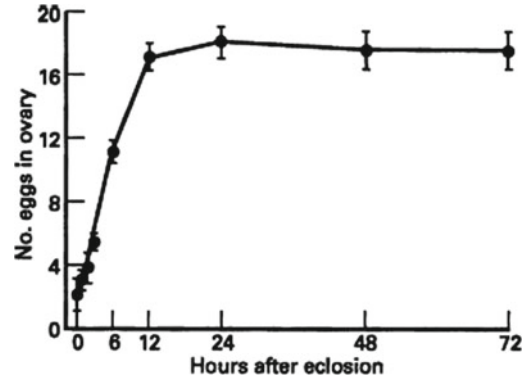


Fig. 2.13 The number of full-sized eggs in the ovaries of *Coccophagus atratus* (Aphelinidae), recorded at various intervals after female eclosion (mean ± SE, $n = 10$). *Source* Donaldson and Walter (1988), reproduced by permission of Blackwell Publishing

optimal 'good world' laboratory conditions are still exceedlingly low, with females only able to lay a maximum of 2–3 eggs a day and rarely more than 50 during a lifetime (Harvey, 2008). It would be interesting to know what conditions facilitate the switching on and off of ovigenesis under both natural and laboratory conditions, and if this is correlated with reproductive traits of the parasitoids being studied.

To measure the rate of ovigenesis in a synovigenic parasitoid in relation to different treatments, expose each of several large cohorts of standardised (e.g., newly emerged) females to a particular environmental condition, e.g., type of diet, temperature level, and follow the cohorts through until the last females die. Each day, dissect part of each cohort and examine the condition of the ovaries in the females, recording the number of mature eggs. The age-specific and average daily rate of ovigenesis (plotted as an ovigenesis schedule) can be compared for the different treatments. A detailed protocol for an investigation of this type, concerned with the effects of different temperatures, may be found in Kajita and van Lenteren (1982).

2.3.6 Motivation to Oviposit

A number of theoretical models indicate that the motivation to oviposit (and to host-feed) depends upon egg load. How does a parasitoid perceive the size of its egg load? Donaldson and Walter (1988), in a detailed study on ovipositional activity and ovarian dynamics in *Coccophagus atratus*, showed that when females were exposed to an abundance of hosts, they deposited eggs within defined bouts of ovipositional activity that were initiated only when the female had accumulated approximately eighteen full-sized eggs (Fig. 2.4a). This finding suggests that egg load, possibly perceived via stretch receptors in the lateral oviducts (Collins & Dixon, 1986), affects the motivation to oviposit.

2.3.7 Spermathecal Complex

The spermatheca (Figs. 2.1, 2.2, 2.4, 2.7, 2.14 and 2.15) is the sperm storage organ of females. Syrphidae, Tachinidae and Pipunculidae have three (Fig. 2.2b; Kozanek & Belcari, 1997), whereas Hymenoptera have only one (Quicke, 1997). In Hymenoptera, the spermatheca is situated at or near the confluence of the lateral oviducts. The spermathecal complex comprises a capsule (the storage vessel or 'spermathecal reservoir'), a gland or pair of glands which may help to attract, nourish and possibly activate sperm, and a muscular duct through which sperm are released (or witheld) as an egg passes along the common oviduct (vagina).

In parasitoid wasps, the spermatheca is noticeably pigmented yellow, dark red or black (a possible adaptation for protecting sperm from the adverse affects of UV light), a useful feature to look out for when dissecting females. Using transmitted light, it is usually possible to observe, at high magnifications, the movement of any sperm present within the capsule. To detect such movement, observations must be made within 5 min of dissecting the recently killed female. Hardy and Godfray (1990) determined whether or not field-caught foraging parasitoids were virgins, by examining the spermatheca of dissected females. They were able to distinguish between empty spermathecae, those containing living sperm (present as a writhing mass) and those containing dead sperm (inadvertently killed by the dissection process). The spermathecae of Pipunculidae are enclosed within the sclerotised base of the ovipositor, and so are difficult to examine and dissect.

Thus far, most empirical attention has focused on egg limitation in parasitoids as a possible impediment to achieving maximum fecundity (egg limitation in parasitoids is discussed in Chap. 1). However, more recently it has been shown that the number of sperm carried by some male parasitoids can also be a limiting factor in

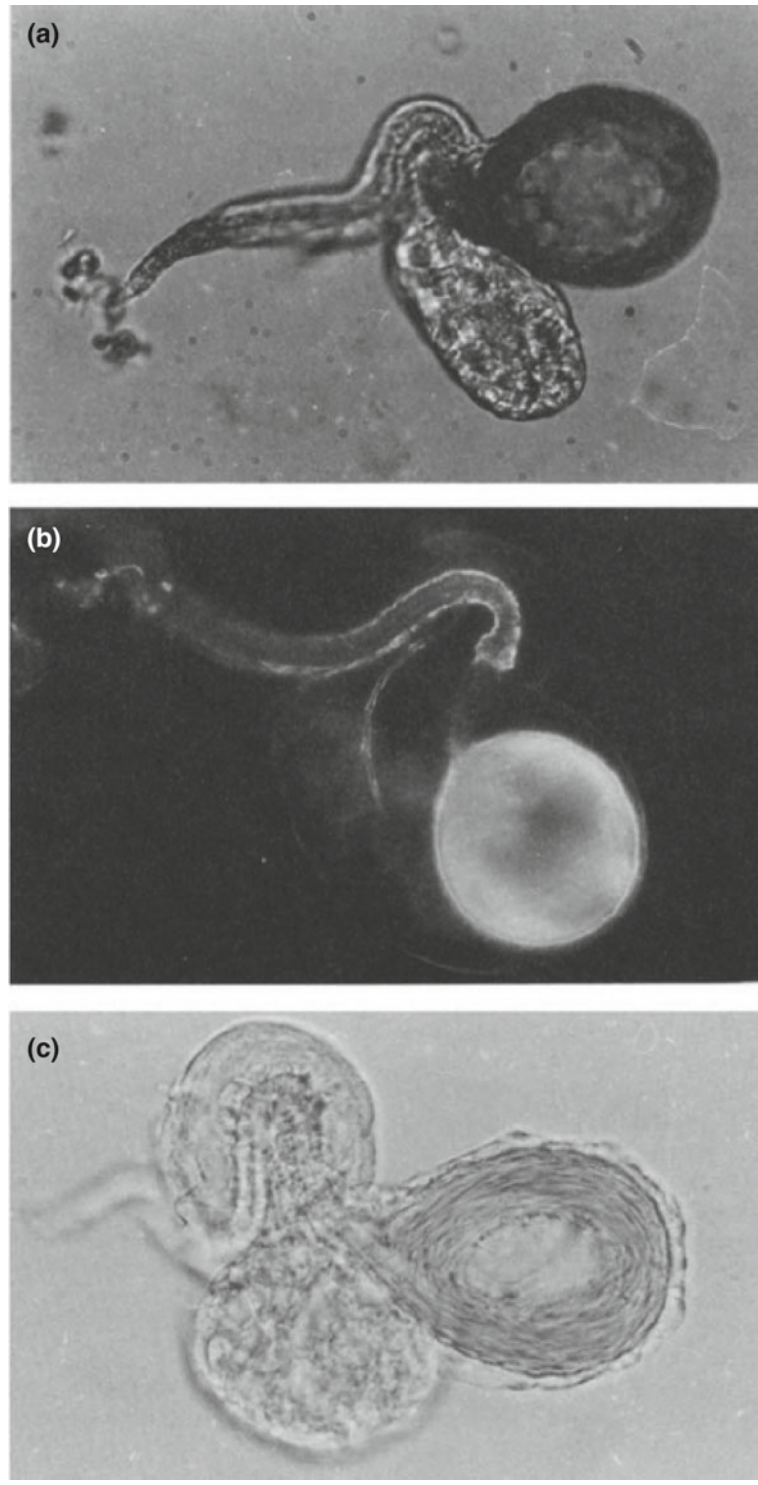


Fig. 2.14 The spermatheca in parasitoid wasps: **a** *Aprostocetus* sp. (Eulophidae) (showing pigmented capsule); **b** *Eurytoma* sp. (Eurytomidae) (capsule and gland united, showing the gland's collecting duct); **c** *Nasonia vitripennis* (Pteromalidae) (showing sperm)

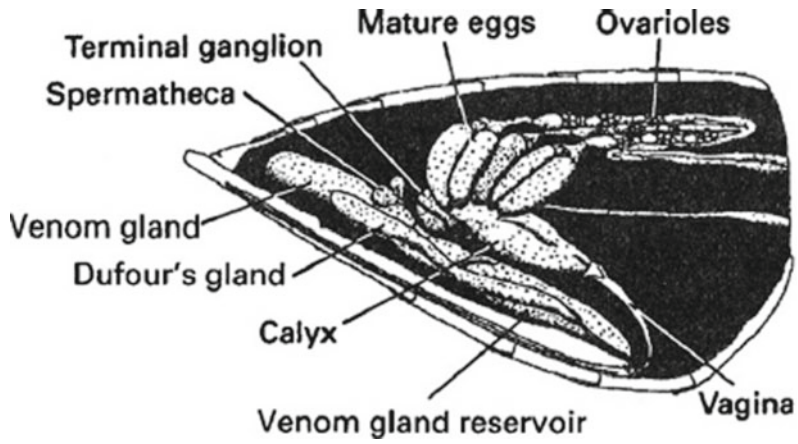


Fig. 2.15 The reproductive system of female *Aphelinus* (Aphelinidae), showing the position of the spermatheca, the venom gland, Dufour's gland, the venom gland reservoir and other structures. *Source* Copland (1976). Reproduced by permission of Pergamon Press

reproduction (Boivin, 2013). Suggested studies on sperm use, limitation, depletion and competition are described in Chap. 4 (Sect. 4.5).

2.3.8 Accessory Glands

In many female insects there are obvious glands, occurring as a pair or two pairs of pouches, associated with the anterior end of the common oviduct (vagina), which are termed accessory or colleterial glands (Figs. 2.4a, e, 2.7 and 2.8) (King & Ratcliffe, 1969; Quicke, 1997). It is generally understood that they produce secretions which coat the egg as it is laid. These glands are present in nearly all chalcidoid parasitoids; different families have different numbers and arrangements (King & Copland, 1969; Copland & King, 1971, 1972a, b, c, d; Copland et al., 1973; Copland, 1976), but hardly anything is known about their function. They have been implicated in the formation of feeding-tubes of host-feeding Hymenoptera (Flanders, 1934) but they seem to be equally developed in species that do not host-feed. Some Torymidae have the largest glands, and *Eupelmus urozonus* (Eupelmidae) has both large glands and enormous extensions from the calyx. Noting the condition of the glands in dissected females under various experimental treatments may be instructive as to their function.

2.3.9 Dufour's (Alkaline) Gland

The Dufour's or alkaline gland (Figs. 2.4a, b, d, 2.15 and 2.16) is well developed in the Hymenoptera. It discharges into the anterior common oviduct at the base of the ovipositor. In parasitoids it is the source of the parasitoid marker substances (pheromones) discussed in Sects. 1.64 and 1.9.4. The Dufour's gland is normally a thin-walled sac containing an oily secretion. It is a long tubular structure in most chalcids but may be extremely small in some braconid wasps, e.g., *Cotesia glomerata*, concealed among the bases of the ovipositor stylets. Gas chromatography can be used to reveal the chemical composition of gland secretion; Marris et al. (1996) showed that in *Venturia canescens* there are quantitative between-strain differences in composition, indicating that different genetic lines produce characteristic cocktails of marker pheromone.

2.3.10 Venom Gland (Acid Gland, Poison Gland)

The venom gland (= acid gland, poison gland), like the Dufour's gland, empties into the base of the ovipositor (Fig. 2.4a, b, d). It is either a simple structure as in Chalcidoidea (Fig. 2.17), a convoluted tubular structure as in Ichneumonidae, or

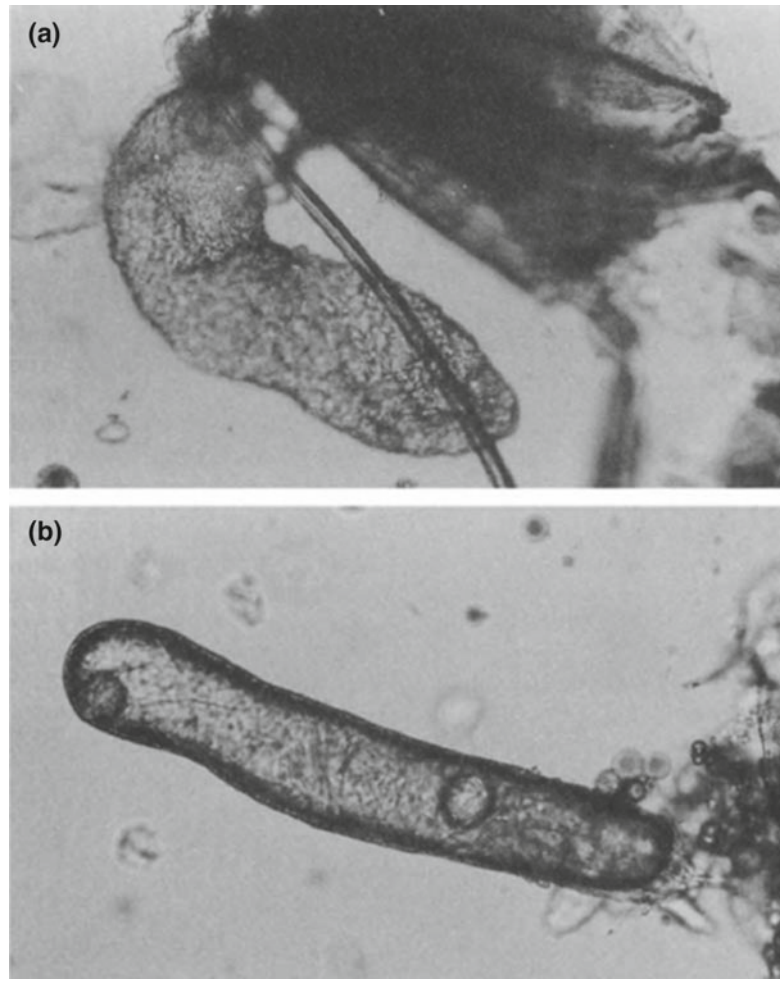


Fig. 2.16 Dufour's or alkaline gland in parasitoid wasps: **a** *Eurytoma* sp. (Eurytomidae); **b** *Colastes* sp. (Braconidae)

a structure of intermediate complexity as in some Braconidae (Fig. 2.17) (see also Quicke, 1997). The venom of some idiobionts induces permanent paralysis, arrested development or death in the host, whereas that of koinobionts induces temporary paralysis or no paralysis at all (see Quicke, 1997, for a discussion of these and other effects). Associated with the venom gland is a reservoir that has muscular walls; the reservoir may have additional secretory functions (Robertson, 1968; van Marle & Piek, 1986). The venom gland has been reported to be a source of viruses or virus-like particles. The structure and function of the venom gland system of hymenopterans has been investigated by several workers (Ratcliffe & King, 1969; Piek, 1986; see also Quicke, 1997, and references contained therein), but there is considerable scope for further investigative work into gland structure and function.

2.4 Male Reproductive System

An example of the reproductive system in male hymenopterans is shown in Fig. 2.18. The system comprises a pair of testes and usually a pair of accessory glands. For further details, see Quicke (1997). The possible role of secretions from the latter in parasitoid mating behavior is discussed in Sect. 4.3.6.

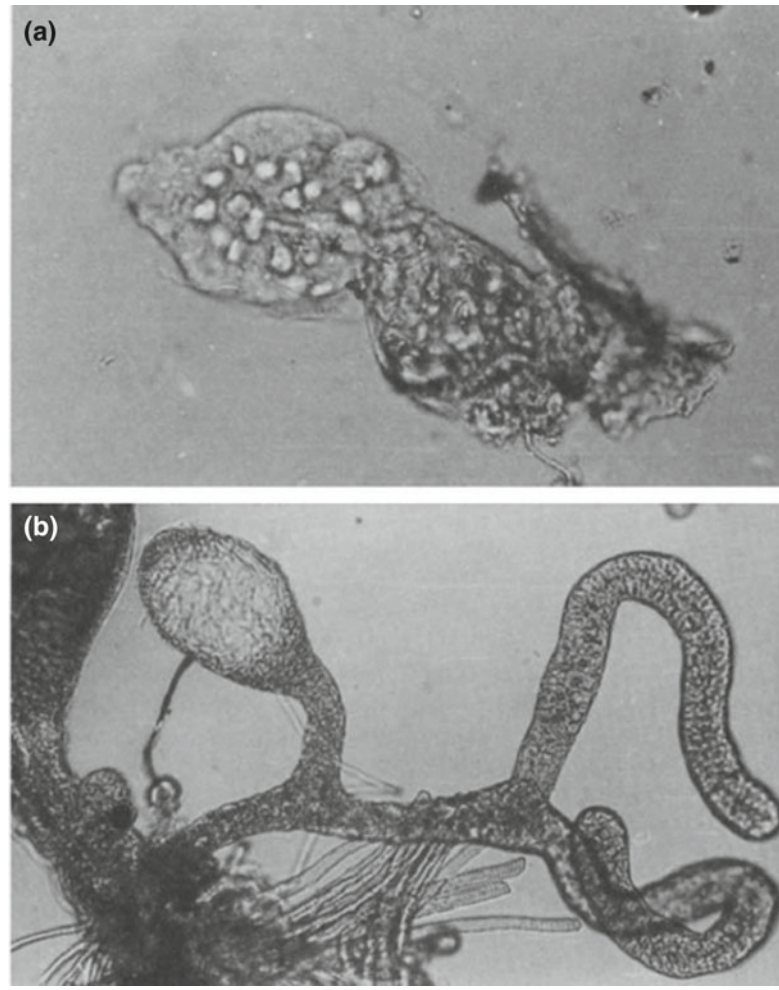


Fig. 2.17 The venom gland in parasitoid wasps: **a** unidentified Mymaridae, showing simple gland and reservoir; **b** *Cotesia* sp., showing more complex, (i.e., bifurcate) gland and reservoir

2.5 Sex Ratio

This aspect of parasitoid and predator biology (including the causes of biased primary and secondary sex ratios), is dealt with in Chaps. 1 (Sect. 1.11) and 3 (Sect. 3.4) (see also Chaps. 4 and 5). The role of *Wolbachia* endosymbionts in biasing sex ratios is touched upon in Chaps. 3, 4, and 6. Some of the biotic and physical factors discussed elsewhere in this chapter (below) may influence secondary sex ratio. For a protocol for studying the effects of (constant and variable) temperatures on progeny sex ratio in parasitoids, see Kfir and Luck (1979).

2.6 Locating Eggs in Hosts

Parasitoid eggs may need to be located, by researchers, in or on hosts for a variety of reasons, including the measurement of fecundity and parasitism (Sects. 2.7.3, 7.2, and 7.3), investigations of parasitoid behaviour (Sects. 1.6.6, 1.9, 1.10, and 1.14) and studies of parasitoid communities (Sects. 6.2.9, and 6.3.5). The degree of difficulty experienced in locating eggs will depend upon factors such as the relative sizes of the host and the parasitoid egg, the amount of fat body tissue, whether the eggs lie within organs or in the haemocoel, the size of other organs, and

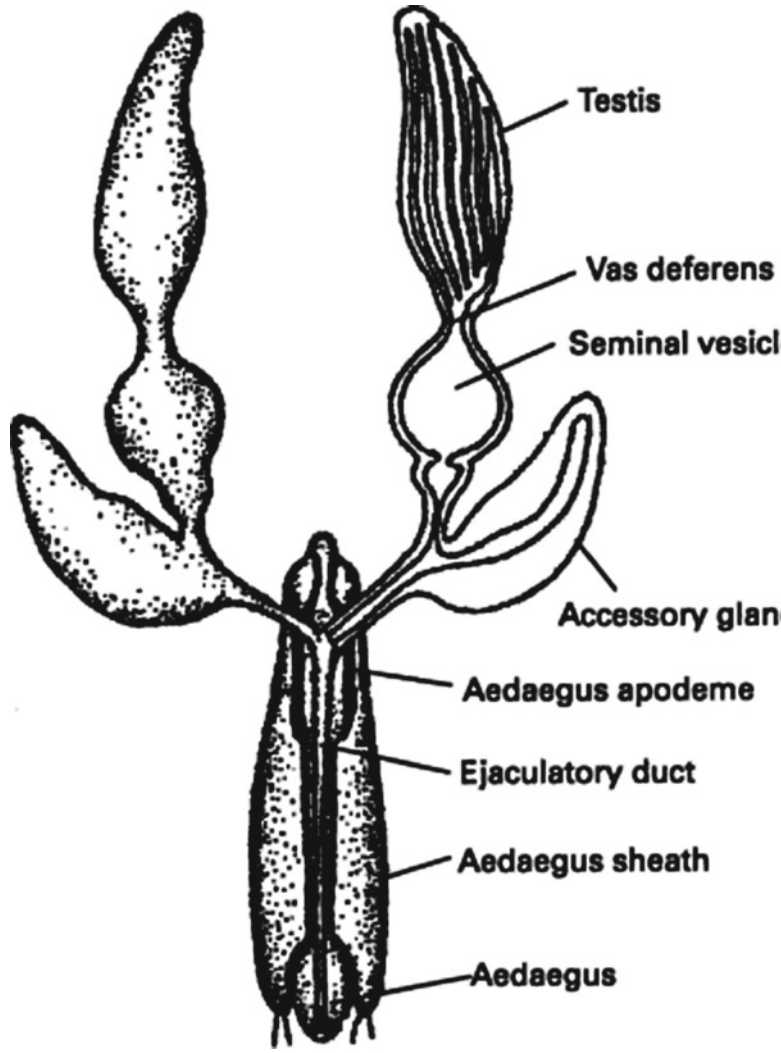


Fig. 2.18 Schematic representation of reproductive system in male Chalcidoidea. *Source* Sanger and King (1971). Reproduced by permission of The Royal Entomological Society of London

the degree of sclerotisation of the host integument (Avilla & Copland, 1987). The eggs of endoparasitoids are generally much more difficult to locate than those of ectoparasitoids.

Preferably, hosts should be killed either: (a) by narcotising them (e.g., using CO_2, ethyl acetate), in which case they should be dissected shortly afterwards, or (b) by placing them in a deep freeze, in which case they can remain dissectable for several months. Attempting to locate eggs in hosts that have been preserved in alcohol is likely to prove very difficult indeed.

If endoparasitoid eggs prove difficult to locate, parasitised hosts should be kept alive long enough for the eggs to swell (i.e., in hydropic species) and/or the first-instar larvae to form, the parasitoid immature stage in either case becoming more easily visible.

2.7 Fecundity

2.7.1 Introduction

The term fecundity refers to an animal's reproductive output, in terms of the total number of eggs produced or laid over a specified period, and should be distinguished from fertility which refers to the number of viable progeny that ensue. From the standpoint of population dynamics, fertility is the more important parameter, as it is the number of progeny entering the next generation. However, because fertility can be relatively difficult to measure (Barlow, 1961), fecundity measurements are often used instead.

A distinction is drawn between potential fecundity and realised fecundity. A species' potential fecundity is usually taken to be the maximum number of eggs that can potentially be laid by females. For example, in the laboratory we might take a strictly pro-ovigenic parasitoid (Sect. 2.3.4), dissect its ovaries at eclosion and then count the number of eggs (all mature) contained within. This number is the insect's potential lifetime fecundity. Synovigenic parasitoids emerge with some immature eggs, so in these insects potential fecundity is the number of mature eggs (the initial egg load) plus the number of immature eggs.

Potential fecundity can be compared with the number of eggs actually laid over the life-span when excess hosts are provided in the laboratory, i.e., lifetime realised fecundity. The figure for lifetime realised fecundity is likely to fall short of the estimate for lifetime potential fecundity. This applies especially to females whose realised fecundity is measured in the field, where female life-span is likely to be significantly shorter (Leather, 1988).

Fecundity is a variable feature of a species, influenced by a range of intrinsic and extrinsic (physical and biotic) factors. The evaluation of a natural enemy for biological control requires a study of the influence of these factors (and of possible interaction effects between certain factors) on potential and realised fecundity, and if possible, fertility. The data can be used in estimating a species' intrinsic rate of increase which is discussed later in this chapter (Sect. 2.11). Fecundity (potential or realised) is also used as a measure of individual fitness in insects (e.g., Hardy et al., 1992; Visser, 1994; Ellers et al., 1998; Roitberg et al., 2001).

When assessing the influence of a particular biotic factor on lifetime realised fecundity, it is important to determine to what extent variation in fecundity can be explained by variation in longevity. For example, take the positive relationship between female size and fecundity. The greater longevity of larger females compared with smaller females could be the sole reason why larger females are more fecund. Females may have the same average daily egg production irrespective of body size, but by living longer, larger females lay more eggs over their life-span (Sandlan, 1979). For a discussion of fecundity–longevity relationships within and among species of predatory coccinellids, see Dixon (2000).

It is possible to obtain measures of realised fecundity without actually counting eggs: Takagi (1985) and Hardy et al. (1992) counted the number of adult offspring produced and took account of the intervening mortality processes, so deriving estimates of the number of eggs originally deposited. In some arthropod predators, such as spiders, it is easy to measure realised fecundity by rearing individual mated females and by removing and rearing out their egg sacs throughout the course of their adult life (Öberg, 2009; Drapela et al., 2011).

2.7.2 Cohort Fecundity Schedules

A (realised) fecundity schedule for a parasitoid or predator species can be constructed by taking a cohort of standardised females (standardised in terms of physiological age, size, and oviposition and sexual experience) and exposing them individually to some chosen set of constant environmental conditions from adult emergence until death. The number of eggs laid per female per day is then plotted, giving the age-specific realised fecundity of the species (Fig. 2.19; see also

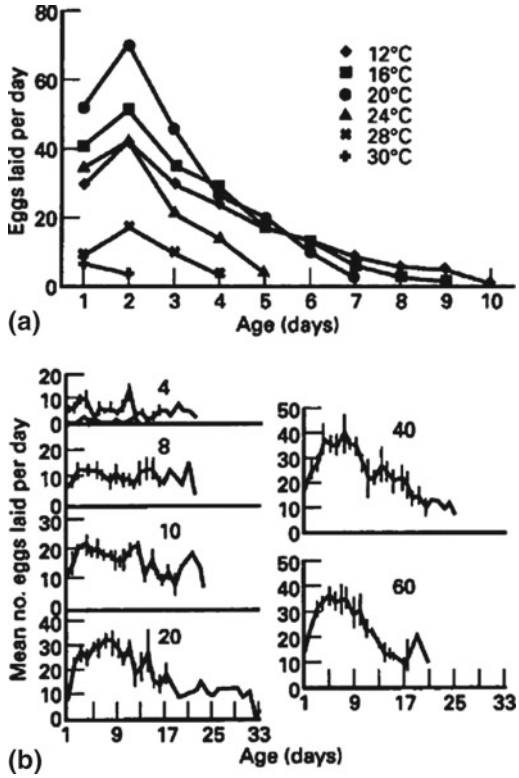


Fig. 2.19 The age-specific fecundity schedule for two parasitoid species: **a** *Aphidius matricariae* maintained at different temperatures and at constant host density conditions (*source* Hag Ahmed, 1989); **b** *Dicondylus indianus* (Dryinidae) maintained at different host densities (4–60) and constant temperature conditions. The plot of host density 2 treatment is shown along with that of the host density 4 treatment (vertical bars = SE). (*Source* Sahragard et al. 1991). Reproduced by permission of Blackwell Verlag GmbH

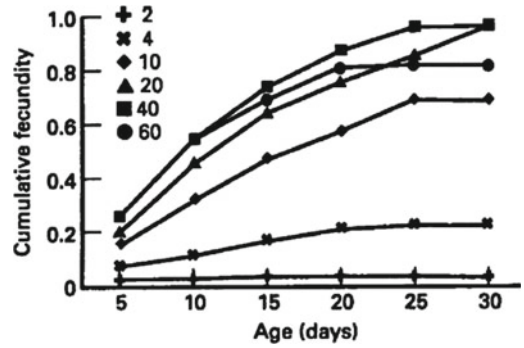


Fig. 2.20 The cumulative realised fecundity of the dryinid wasp *Dicondylus indianus*, measured over the lifetime of females, at different levels of host availability. Fecundity is expressed as the proportion of the highest mean total number of eggs laid by females of any one treatment, this total representing the maximal fecundity that could be realised. *Source* Sahragard et al. (1991). Reproduced by permission of Blackwell Verlag GmbH

Fig. 2.65). The data obtained from the experiment can also be used to calculate both the lifetime realised fecundity of the species (used by evolutionary ecologists as a measure of fitness, see Roitberg et al., 2001), and the average daily oviposition rate (lifetime realised fecundity divided by the average longevity). Using the same data, the cumulative realised fecundity of the parasitoids can also be plotted against either female age (Fig. 2.20) or cumulative degree-days (Minkenberg, 1989) (Sect. 2.9.3). It is expressed as the proportion of the highest mean total number of eggs laid by females of any one treatment (e.g., temperature or host density treatment), this total representing the maximal fecundity realisable by females. The usefulness of the cumulative realised fecundity measure is that it tells us to what extent parasitoids achieve their maximum lifetime fecundity (~fitness) under particular conditions, and allows easier comparison of the effects of different treatments. Using the data from a fecundity schedule, the parameters m_x (age-specific fecundity) and l_x (age-specific survival) can be used in the calculation of the intrinsic rate of increase (r_m) of the parasitoid population (Sect. 2.11). If fecundity schedules are constructed for cohorts held under different host or prey availability regimes, the number of hosts or prey parasitised or eaten can be recorded and the data used to plot age-specific and lifetime functional responses (the numbers parasitised or eaten *versus* the numbers available; Sect. 1.14), as was done by Bellows (1985a).

An important consideration when using the aforementioned experimental design is that as time goes on, the data are limited to progressively fewer females. To obtain fecundity data that are statistically meaningful, particularly data for the latter part of adult life, a very large starting density of parasitoid or predator females may be required. This, however, may increase

the investigator's workload to an unacceptable level.

In most parasitoids and in predators, the realised fecundity schedule (and also the ovigenesis schedule, see Sect. 2.3.5) will show a rise in the number of eggs produced or laid per day until a maximum rate of productivity is reached. Thereafter a gradual decrease occurs until reproduction ceases altogether at or shortly before the time of death (see Kindlmann et al., 2001, for a discussion of this 'triangular fecundity function') (Fig. 2.19) If there is a period of post-reproductive life, it is usually very short (see Jervis et al., 1994, for exceptions). Fecundity schedules vary between species, depending on the reproductive strategies of the insects, e.g., strict pro-ovigeny and different degrees of synovigeny (Sect. 2.3.4). As described below, environmental factors (temperature, humidity, photoperiod, light quality, light intensity, host or prey availability) modify these patterns in a number of ways, and ideally the role of each factor in influencing the schedule ought to be investigated separately. This, however, may not be practicable, in which case the usual procedure is to expose a predator or parasitoid to an excess of prey or hosts (replenished or replaced daily), at a temperature, a relative humidity, or a light intensity similar to the average recorded in the field (Dransfield, 1979; Bellows, 1985a).

2.7.3 Effects of Biotic Factors on Fecundity

2.7.3.1 Host Density (Parasitoids)

If fecundity schedules are constructed for a parasitoid species over a range of host densities, females will be found to lay on average more eggs per day at higher host densities than at low densities (Fig. 2.21). Also, the lifetime pattern of oviposition, i.e., the shape of the curve, varies with host density. There may be a shift in the fecundity schedule, with wasps concentrating oviposition into the earlier part of adult life (Fig. 2.19b). At high host densities, hosts are more readily available for the wasps to attack, whereas at low densities oviposition rates are lower because the wasps have to search a greater area (and probably for a longer period of time), so expending energy that might otherwise be used in ovigenesis (Sahragard et al., 1991). Venkatesan et al. (2009) reported that in the laboratory a parasitoid:host ratio of 1:1 resulted in maximum fecundity and number of progenies, and increasing the densities of either of these two had an inverse effect on oviposition. As far as lifetime fecundity is concerned, the relationship with host density is either a curvilinear one, resembling a Type 2 functional response (defined in Sect. 1.14), or a sigmoid one, resembling a Type 3 functional response.

A difficulty that may arise when using low host densities is ovicide, i.e., the removal of eggs from parasitised hosts, although the number of (ecto)-parasitoid species that practice ovicide is considerably smaller than the number of predator species that do so. Among parasitoids, ovicide has been observed in several families of primary parasitoids and hyperparasitoids (Strand & Godfray, 1989; Mayhew, 1997; Netting & Hunter, 2000; Pérez-Lachaud et al., 2004; Nakashima et al., 2016). Predaceous females of chrysopid lacewings are well known for eating their own eggs in laboratory cultures (Principi & Canard, 1984), as are some coccinellids (Michaud, 2003). Where cannibalism is suspected, video-recording techniques may help in determining the number of eggs lost in fecundity experiments.

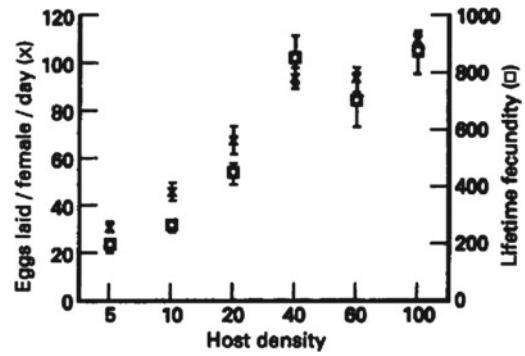


Fig. 2.21 The relationship between fecundity (measured as both the mean number of eggs laid per day and the total number of eggs laid over adult life) and host availability in the parasitoid *Aphidius smithi* (Braconidae) (Error bars = SE). Based on data taken from Mackauer (1983)

2.7.3.2 Food Consumption

Non-predaceous Females

The females of many parasitoid and some predator species (e.g., *Chrysoperla carnea* (Chrysopidae) and adults of all aphidophagous Syrphidae) feed as adults solely on materials such as honeydew, nectar and pollen (Chap. 8), and consume substitute foods such as diluted honey in the laboratory (Chap. 8; see also Benelli et al., 2017). Even arthropod taxa that are often to be considered as wholly predaceous, such as spiders, often consume pollen or nectar to supplement dietary prey (Taylor & Foster, 1996; Taylor & Pfannestiel, 2009; Kuja et al., 2012). Females that are either deprived of food or experience a reduced intake (but are given water) lay fewer eggs or no eggs at all. Some non-host/prey foods have a more beneficial effect on fecundity than others (Krishnamoorthy, 1984; Principi & Canard, 1984; Wratten et al., 2003; Heimpel & Jervis, 2004; Jervis et al., 2004; Heimpel, 2019).

For an experimental investigation into the effects of adult nutrition on the fecundity schedule of a parasitoid to be ecologically meaningful, the effects of food provision need to be considered in the light of variations in host availability. This is done by taking a cohort of standardised females and providing the insects with one of a range of host densities (see Host Density, above) and with a chosen diet for the duration of their lives, the hosts and food being replenished daily. If the effects upon ovigenesis of combined host deprivation/food provision are to be investigated, then, obviously, hosts are not provided to one set of females. One likely effect of providing food to females is that, at low host densities, females maintain a higher rate of oviposition than they can when deprived of food. As far as the effects of food provision on lifetime fecundity are concerned, it will be necessary to carry out a statistical analysis to show whether or not any improvement in lifetime fecundity brought about by feeding is simply a result of an increase in longevity and not an increase in the daily rate of ovigenesis (Sect. 2.8.3).

Predaceous Females

We would expect the fecundity of predaceous females to be strongly influenced by prey availability. This relationship was modelled in a simple way by Beddington et al. (1976) and Hassell (1978). If it is assumed firstly that some of the food assimilated by the female needs to be allocated to maintenance metabolism (and will therefore be unavailable for ovigenesis), and secondly that there is insufficient carry-over of food reserves from larval development for the laying of any eggs (i.e., synovigeny-anautogeny), then there will be a threshold prey ingestion rate, c, below which reproduction ceases, but above which there is some positive dependence between fecundity F and ingestion rate I. If it is assumed thirdly that this relationship is linear, then (Beddington et al., 1976):

$$F = \frac{\lambda}{e}(I - \mathrm{c}) \tag{2.1}$$

where e, λ and c are constants; e is the average biomass per egg. There is empirical support for this model (Mukerji & LeRoux, 1969; Mills, 1981; Fig. 2.22). In Mills' (1981) experiment five feeding levels were used, the daily ration of individual females corresponding to between 1 and 2 times the average female weight.

To express fecundity in terms of prey density, we first assume ingestion rate to be proportional to the number of prey eaten, N_a, such that:

$$I = kN_a \tag{2.2}$$

where k is a constant which depends upon the biomass (size) of each prey. Combining Eqs. 2.1 and 2.2 with the simplest functional response model, Holling's (1966) disc equation (Sect. 1.14), gives:

$$F = \frac{\lambda}{e}\left[\frac{ka'N}{1 + a'T_hN} - c\right] \tag{2.3}$$

This model predicts that fecundity will rise at a decreasing rate (i.e., will decelerate) towards an upper asymptote as prey density increases, in the

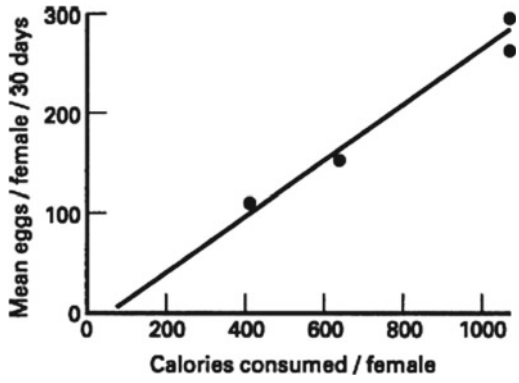


Fig. 2.22 Fecundity as a function of ingestion rate in the predatory pentatomid bug *Podisus maculiventris*. *Source* Beddington et al. (1976), who used data from Mukerji and LeRoux (1969). Reproduced by permission of Blackwell Publishing

manner of the Type 2 functional response (Sect. 1.14), and also that the curve will be displaced forwards along the prey axis, i.e., away from the origin. There is empirical support for this relationship, both from laboratory studies (Dixon, 1959; Ives, 1981; Matsura & Morooka, 1983 (Fig. 2.23a, b) and from field studies (Wratten, 1973; Mills, 1982) (Fig. 2.24a, b). Anautogenous, obligate host-feeding parasitoids will have a similar fecundity/host density curve. In autogenous predators, however, ovigenesis and oviposition can occur without the female first feeding on prey, so the curve of these insects will not be displaced along the prey axis.

In the bug *Anthocoris confusus*, the viability (fertility) of eggs also varies with prey availability (Evans, 1973; Beddington et al., 1976). This relationship may be due to the female allocating less biomass per developing egg at lower prey densities, i.e., *e* in Eq. (2.1) is not a constant (Beddington et al., 1976). In the Western black widow spider, however, urban-living spiders were in worse physical condition, laid fewer eggs, and invested less metabolic resources per egg than desert-living widow spiders despite greater prey availability in the former habitat. Therefore, resource abundance is not always a reliable indicator of fecundity and fitness in predatory arthropods (Johnson et al., 2012).

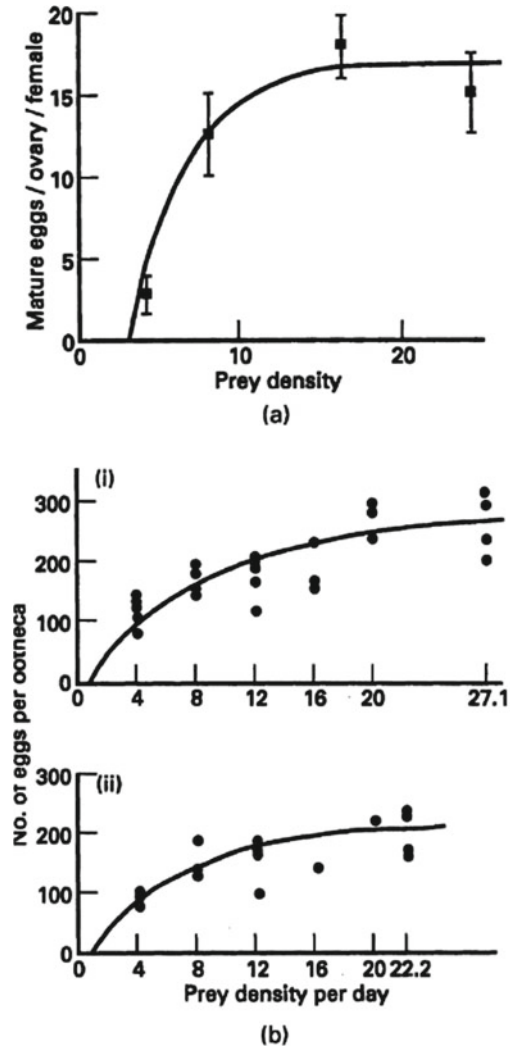


Fig. 2.23 Fecundity as a function of prey density (functional response): **a** in the coccinellid beetle *Adalia decempunctata* (*source* Beddington et al., 1976, who used data from Dixon, 1959); **b** in the mantid *Paratenodera angustipennis:* (i) first ovipositions, (ii) second ovipositions (oötheca = egg mass). Below the intercept of the curve (fitted by eye) with the prey axis, the insects allocate matter to maintenance processes only (*source* Matsura & Morooka, 1983). **a** Reproduced by permission of Blackwell Scientific Publications Ltd; **b** by permission of Springer Verlag

There are also grounds for questioning the assumption that *k* in Eq. (2.2) is a constant (Beddington et al., 1976). If this assumption is correct, then the relationship between fecundity

and the number of prey actually killed will be rectilinear, which is the case for *Coccinella undecimpunctata aegyptiaca* (Fig. 2.25). However, as noted in Chap. 1 (Sect. 1.14), when the rate of encounter with prey is high, some predators consume proportionately less of each prey item. This behaviour will alter the shape of the fecundity *versus* prey killed curve, from rectilinear to curvilinear (Beddington et al., 1976). The shape of the fecundity *versus* prey density curve will also be altered, having an earlier 'turnover' point and also being more 'flat-topped' (Beddington et al., 1976).

Supplying predators with non-prey foods together with prey might lower the ingestion rate threshold, since less of the prey biomass assimilated by the female needs to be allocated to maintenance metabolism. If so, the fecundity–prey density curve of an anautogenous species will be shifted backwards along the prey axis, i.e., towards the origin. The shape of the curve is also likely to be altered.

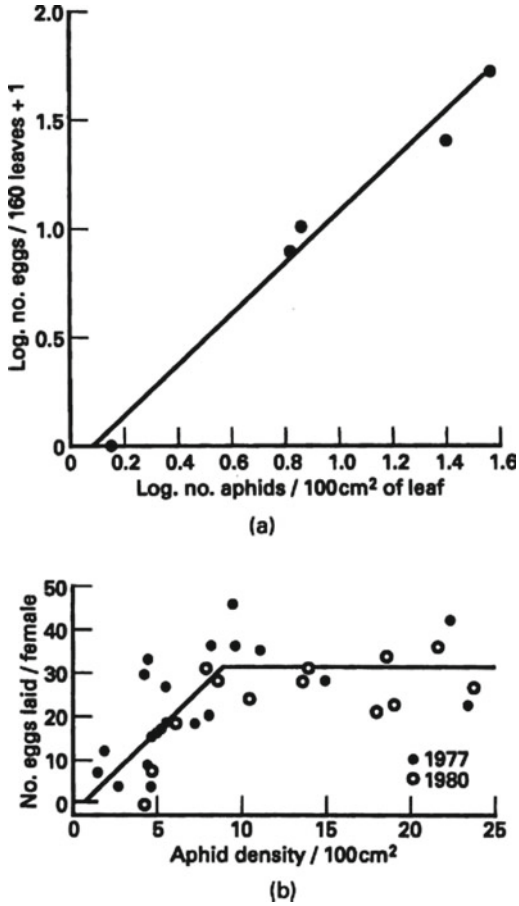


Fig. 2.24 Fecundity as a function of prey density: **a** relationship between logarithm of number of eggs laid by the coccinellid *Adalia bipunctata*, and logarithm of density of aphids in the field (data from Wratten, 1973); **b** relationship between number of eggs laid per adult *Adalia bipunctata* and aphid density in the field (*source* Mills, 1982). **a** Reproduced by permission of Blackwell Scientific Publishing Ltd; **b** by permission of The Association of Applied Biologists

2.7.3.3 Prey and Host Quality

Prey quality is likely to affect fecundity, as has been shown for Coccinellidae, Carabidae, Anthocoridae, and host-feeding Aphelinidae (Hariri, 1966; Blackman, 1967; Hodek, 1973; Wilbert & Lauenstein, 1974; Spieles & Horn, 1998; Evans et al., 1999; Venzon et al., 2002). Some coccinellids and carabids are unable to reproduce at all if confined to a diet of certain prey species (Hodek, 1973; Spieles & Horn, 1998; Evans et al., 1999). Among parasitoids, *Goniozus nephantidis*, a larval parasitoid of *Opisina arenosella*, laid the most eggs and produced the most progeny on largest caterpillars of both the natural and a factitious host species (Shameer et al., 2002).

Blackman (1967) found that adults of the coccinellid beetle *Adalia bipunctata* fed on *Aphis*

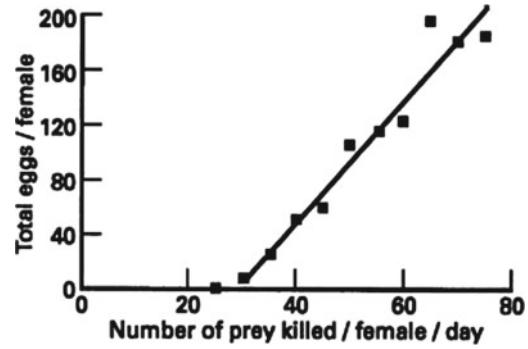


Fig. 2.25 The relationship between fecundity and prey consumption rate in *Coccinella undecimpunctata aegyptiaca*. *Source* Beddington et al. (1976), who used data from Hodek (1973). Reproduced by permission of Blackwell Publishing

fabae during both larval development and adult life were less than half as fecund as those fed on *Myzus persicae*. Also, their eggs were smaller and less fertile. By carrying out another experiment in which adult beetles were fed on the opposite prey species to that fed upon by the larvae, Blackman (1967) tested whether the prey species given to larvae affected the fecundity of the adult. It did not: fecundity depended strongly upon the species fed upon by the adult. Similarly, Sigsgaard et al. (2001) tested growth, survival and fecundity of the dwarf spider *Atypena formosana* (Linyphiidae) fed on different prey species, and found that the spiders performed significantly better and produced more progeny when reared on some prey species than others. However, it is not clear from either study whether the effects of prey availability were monitored. The results of a study by Hariri (1966) are shown in Fig. 2.26. Evans et al. (1999) showed that when two species of predatory coccinellids are exposed to limited numbers of their preferred aphid prey, fecundity is enhanced if females are supplied with an additional prey species (a weevil), despite the fact that females given weevils alone cannot produce eggs. In predators such as coccinellids the pre-oviposition period may be either shortened or prolonged, depending on the prey species fed upon by the female (Hodek, 1973).

2.7.3.4 Consumption of Food Supplements and Substitutes (Predaceous Females)

As we have suggested, fecundity is very likely to vary with the availability (and the quality) of plant-derived and other non-host/prey foods (especially so in the case of species having a high requirement for such nutrient input), taken either as supplements (when prey are available) or as substitutes (when prey are absent). Several predators have been shown to have a higher rate of egg production when given non-host foods as a supplement (e.g., Cocuzza et al., 1997a; Crum et al., 1998), but except for some artificial diets, non-prey foods are a poor substitute for prey materials, in terms of their effects on fecundity (e.g., Cocuzza et al., 1997a; Evans et al., 1999) (this may not apply to predator species whose diet is normally comprised largely of plant materials). In *Aphytis melinus* the benefit, to fecundity, of host-feeding cannot be realised unless females also feed on sugar (Heimpel et al., 1997a; Chap. 8).

2.7.3.5 Mutual Interference

Mutual interference between female parasitoids results in a reduction in individual searching efficiency (Sect. 1.15.3) which will result in a reduction in the rate of oviposition, i.e.,

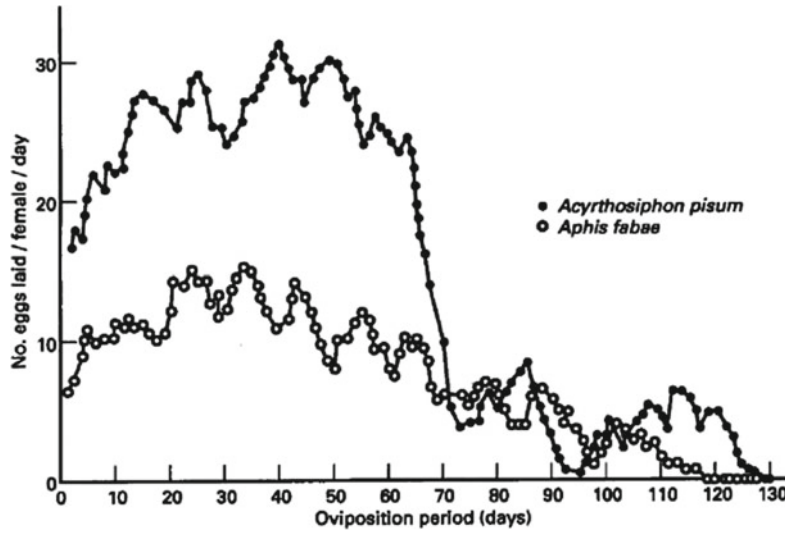


Fig. 2.26 Fecundity of the coccinellid beetle *Adalia bipunctata* maintained on different prey species, *Acyrthosiphon pisum* and *Aphis fabae*. *Source* Hariri (1966). Reproduced by permission of W. Junk Publishers

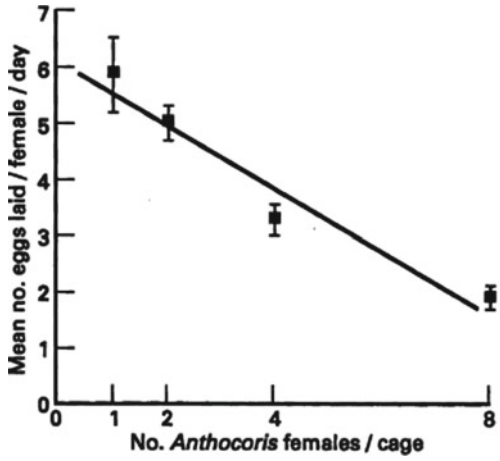


Fig. 2.27 The relationship between fecundity and predator density in the predator *Anthocoris confusus*. There was a decline in fecundity despite aphid prey density being high at all times, i.e., the cause of the decline was mutual interference, not exploitation of prey. *Source* Evans (1976). Reproduced by permission of Blackwell Publishing

fecundity. In the predator *Anthocoris confusus* fecundity declined with increasing adult density, despite the fact that prey density was high at all times and was unlikely to limit egg production through prey exploitation (Evans, 1976; Fig. 2.27). To determine whether mutual interference was a result of confining predators in his experimental cages, Evans (1976) measured fecundity in relation to predator density in females in a large cage within which they were free to move from plant to plant. A significant decrease in fecundity with increasing predator density was still recorded.

Mutual interference, and therefore interference-mediated reductions in fecundity, cannot be assumed to occur in all predators. For example, Hattingh and Samways (1990) found no evidence for mutual interference in adults of three species of *Chilocorus* (Coccinellidae). Feeding rate did not decrease and dispersal did not increase with increasing beetle density. Among parasitioids, mutual interference between adult females during the host- and clutch-guarding phases in the bethylid *G. nephantidis* led to considerable reductions in the number of offspring produced, even though each female was experimentally provided with a host (Sreenivas & Hardy, 2016).

2.7.3.6 Female Body Size

In the laboratory, lifetime fecundity, and also reproductive correlates such as ovariole number and egg load (the latter usually recorded either at or shortly after eclosion), have been shown to increase with increasing body size within species (Fig. 2.28) (e.g., Sandlan, 1979; Mani & Nagarkatti, 1983; Ernsting & Huyer, 1984; Nealis et al., 1984; Scott & Barlow, 1984; Waage & Ng, 1984; Bellows, 1985b; Juliano, 1985; Liu, 1985a; Takagi, 1985; Collins & Dixon, 1986; Opp & Luck, 1986; van Vianen & van Lenteren, 1986; Banks & Thompson, 1987a; Moratorio, 1987; van den Assem et al., 1989; Heinz & Parrella, 1990; O'Neill & Skinner, 1990; le Masurier, 1991; Hardy et al., 1992; Rosenheim & Rosen, 1992; Sequeira & Mackauer, 1992b; Croft & Copland, 1993; Zheng et al., 1993b; King & King, 1994; Visser, 1994; Weisser et al., 1997; Ellers et al., 1998; Olson & Andow, 1998; Taylor et al., 1998; Harvey et al., 2000b, 2001; Mills & Kuhlmann, 2000; Martínez-Martínez & Bernal, 2002; Pexton & Mayhew, 2002). There are, however, a few exceptions to this pattern (e.g., Rotheray & Barbosa, 1984; Bigler et al., 1987; Corrigan & Lashomb, 1990; Visser, 1994; Coombs, 1997; Mills & Kuhlmann, 2000).

Some of the restricted number of field studies conducted to date have demonstrated a positive intraspecific relationship between body size and fecundity (Visser, 1994; Kazmer & Luck, 1995; Ellers et al., 1998, 2001; Lauzière et al., 2000; Bezemer & Mills, 2003; Kasamatsu & Abe, 2015; Wang & Keller, 2020).

In *Nasonia vitripennis* the slope of the egg load–body size relationship recorded 48 and 72 h after emergence was steeper in unfed females than in fed ones (Rivero & West, 2002). This result could explain why at least some researchers have recorded a difference between the size–fecundity plots of field and laboratory populations of a species. Small-sized wasps emerge with smaller fat reserves, and so rely more than large wasps upon obtaining food to fuel ovigenesis (Rivero & West, 2002). Because in the field

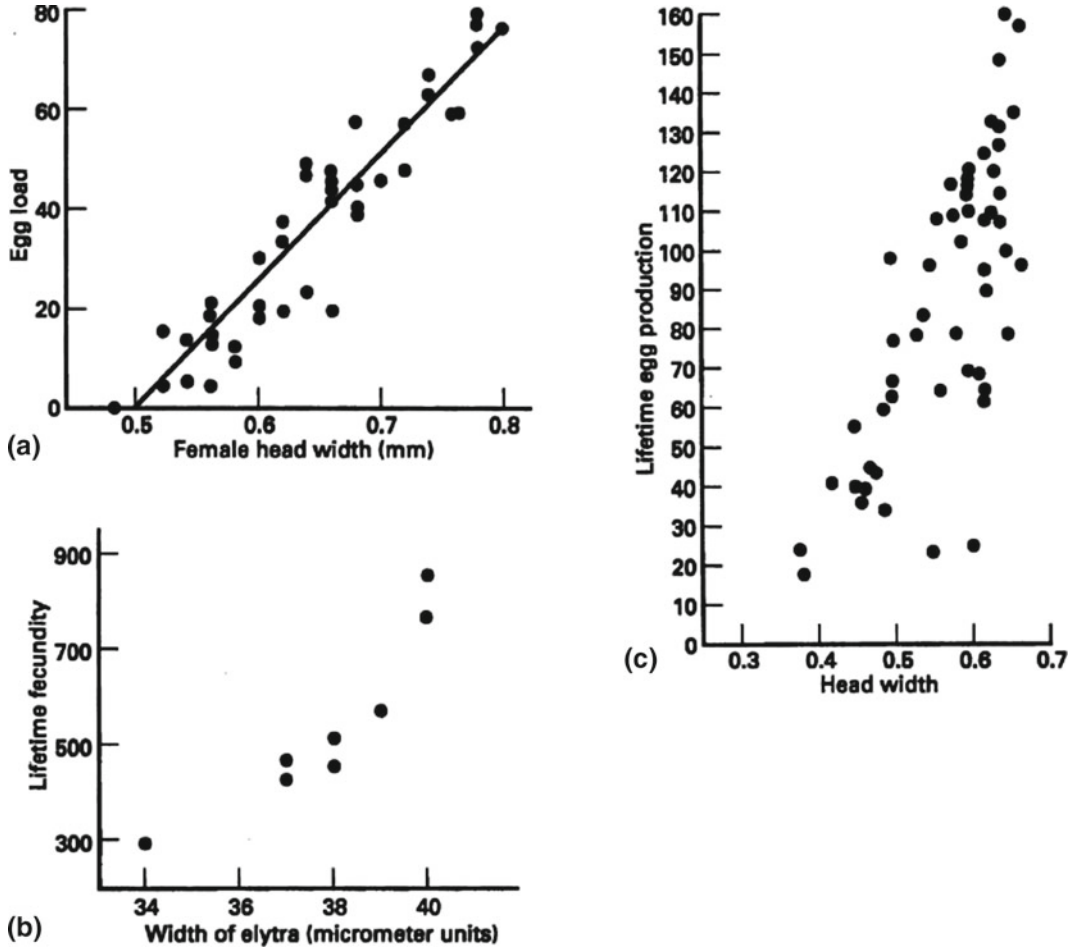


Fig. 2.28 The positive correlation between fecundity measures (egg load, lifetime fecundity) and body size in females: **a** egg load in *Nasonia vitripennis* (*source* O'Neill & Skinner, 1990); **b** lifetime fecundity in *Notiophilus biguttatus*; elytra width is expressed in micrometer units (100 units = 5.0 mm) (*source* Ernsting & Huyer, 1984); **c** lifetime fecundity in *Lariophagus distinguendus* (Pteromalidae) (*source* van den Assem et al., 1989). **a** Reproduced by permission of The Zoological Society of London; **b** by permission of Springer Verlag; **c** by permission of E.J. Brill (Publishers) Ltd

food can often be limiting (Heimpel & Jervis, 2004), small-sized wasps suffer disproportionately in terms of their realised fecundity.

In some species, the relationship between fecundity and body size correlates over only part of the size range, with fecundity reaching a maximum in insects above a threshold size, e.g., *Aphidius ervi* (Sequeira & Mackauer, 1992b). It is therefore important, in experiments, to provide the complete field range of host sizes to parasitoids, so as to avoid obtaining a misleading impression of the 'true' size–fecundity relationship. In predators, larger females have a shorter pre-oviposition period than smaller ones (Zheng et al., 1993b), and this may contribute to their higher lifetime fecundity.

Body size is usually measured in terms of the width or length of some body part, such as the head, thorax, or hind tibia. Some authors also assess size by dry or fresh body weight. More recently, a new estimate of body condition has been devised, which combines linear and volumetric parameters of body size into a single scaled mass measurement (Peig & Green, 2009).

Body size, mass or condition is influenced, within species, by:

1. Larval feeding history, i.e., prey availability, host size, host species during development, quality of host diet (note that this includes plant resistance effects, i.e., bottom-up effects), clutch size, superparasitism (Dixon, 1959; Russel, 1970; Hodek, 1973; Dransfield, 1979; Sandlan, 1979; Cornelius & Barlow, 1980; Beckage & Riddiford, 1983; Principi & Canard, 1984; Scott & Barlow, 1984; Waage & Ng, 1984; Juliano, 1985; Liu, 1985a; Sato et al., 1986; Eller et al., 1990; Bai & Mackauer, 1992; Harvey et al., 1993, 1994, 2000b; Zheng et al., 1993a, b; van Dijk, 1994; Bernal et al., 1999; Martínez-Martínez & Bernal, 2002) (Fig. 2.29).
2. The temperature during larval development (Ernsting & Huyer, 1984; Nealis et al., 1984; van Dijk, 1994) (Fig. 2.30).

If an experiment, for whatever purpose, requires females to be of different sizes/fecundities, by far the simplest way of sorting insects according to size is to measure the parasitoids or predators when they are pupae (pupal and adult size being strongly correlated), so avoiding any difficulties and/or harmful side effects associated with handling the adults.

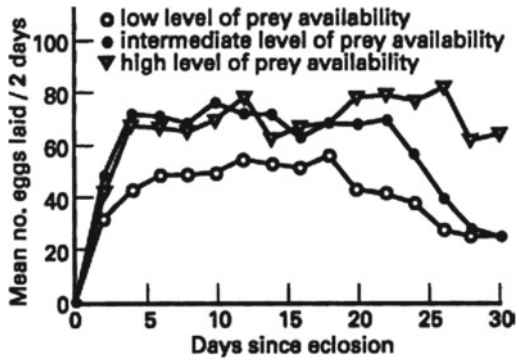


Fig. 2.29 The effect of larval feeding history on fecundity in the lacewing *Chrysoperla carnea*. The data points indicate the average number of eggs laid, per 2-day period, of females provided with different levels of prey availability as larvae. Zheng et al. (1993b) showed that when lacewing larvae are fed fewer prey than they can potentially consume, they develop into smaller and less fecund adults than when they are given an overabundance of prey. Adults of *C. carnea* are non-predaceous, feeding on nectar, pollen and honeydew, and fecundity is also affected by consumption of these foods. Therefore, female fecundity is determined both by larval feeding history and by adult food consumption. *Source* Zheng et al. (1993b)

2.7.3.7 Mating

Female predators and dipteran parasitoids, if they are either unmated or sperm depleted, lay much smaller numbers of eggs (e.g., very few in coccinellids, Dixon, 2000, half as many in the bug *Podisus maculiventris*, De Clercq & Degheele, 1997) or none at all. Eggs, if laid, are infertile. To achieve their full reproductive potential, females of some species may need to mate several times (Sem'yanov, 1970; Ridley, 1988). By contrast, if a female arrhenotokous hymenopteran parasitoid lacks sperm for whatever reason, she can lay viable (male) eggs, so her fecundity should not be affected by mating. Mating was found not to affect egg load in the braconid wasp *Phanerotoma franklini*, but in this case mating was not

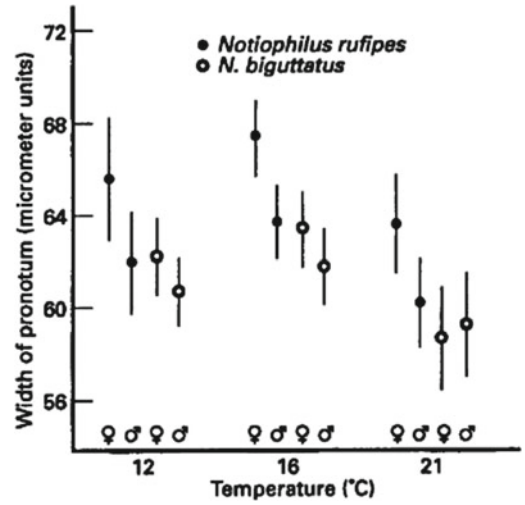


Fig. 2.30 The effect of temperature during larval development upon adult size (as measured by pronotum width) in two species of carabid beetle and for males and females. Means and the corresponding 95% confidence limits are shown, expressed in micrometer units (100 units = 2.5 mm). The data show a decline in adult size at either side of an optimum temperature for total biomass production. The effects upon size are translated into variations in fecundity. *Source* Ernsting and Huyer (1984). Reproduced by permission of Springer Verlag

confirmed to have occurred in all cases (Sisterton & Averill, 2002).

In experiments aimed at testing for the effects of mating, it is essential to establish that mating really has taken place. Caging females with a male is no guarantee that the insects have either engaged in mating behaviour or that the females have been inseminated. Some species or arthropod groups are easier to observe mating than others. For instance, some parasitoid wasps and spiders readily mate when males are placed with females, whereas in other species females repeatedly resist mating attempts made by males or mating takes place out of sight within the confines of cocoons (Chap. 4). It is therefore imperative to visually observe successful mating to ensure that it has taken place. If an effect of mating upon fecundity is found, the question arises, in the case of females, as to whether ovigenesis has been enhanced because of the nutrient contribution made by the male, in the form of sperm or spermatophore.

2.7.3.8 Field Predation

Predator-induced mortality of adult parasitoids and predators may cause realised fecundity to be reduced well below the level achieved under laboratory conditions. The extent of the reduction can be estimated by marking and releasing individuals and cohorts of parasitoids and predators, recording predation events (Heimpel et al., 1997b), and then relating the field survivorship data to the natural enemies' fecundity schedule recorded under optimum laboratory conditions.

2.7.4 Effects of Physical Factors on Fecundity

2.7.4.1 Temperature

The rate of egg production, and hence the age-specific and the lifetime fecundity of predators, and parasitoids, will vary in relation to temperature (van Lenteren et al., 1987; Braman & Yeargan, 1988; Miura, 1990; Li & Jackson, 1996; Hentz et al., 1998; Ellers et al., 2001; Pervez & Omkar, 2004; Pandey & Tripathi, 2008; Murthy et al., 2008; Aung et al., 2010; Watt et al., 2016; Fig. 2.31). The influence of temperature on the fecundity schedule of a natural enemy species can be investigated by taking cohorts of standardised females and exposing each of them to one of a range of temperatures for their lifetimes. Females of all the cohorts are exposed to the same conditions of host/prey, food and water availability (hosts and prey need to be replaced daily), humidity and photoperiod, etc. A constant humidity will probably be the most difficult of all these factors to maintain. Temperature may influence the rate of prey consumption (Mills, 1981; Pickup & Thompson, 1990), so temperature-related variation in prey consumption should be looked for.

The effect of temperature upon egg load in a synovigenic insect can be investigated by following the protocol, used for *Aphytis* parasitoids, of Rosenheim and Rosen (1992). Parasitoid pupae are isolated, and adults, when they emerge, are kept with a supply of food (honey), at each of a range of temperatures for 24 h. The adults are then dissected and the numbers of mature eggs they contain are counted. The results

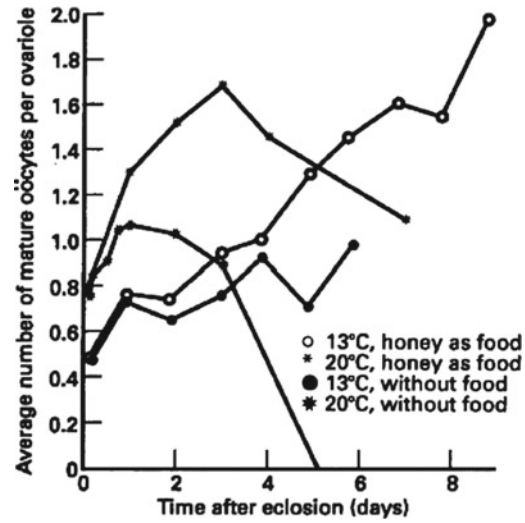


Fig. 2.31 The number of mature oöcytes per ovariole in the parasitoid *Encarsia formosa* (Aphelinidae) kept for several days after eclosion without hosts, either without food or on a diet of honey, at two different temperatures. *Source* van Lenteren et al. (1987). Reproduced by permission of Blackwell Verlag GmbH

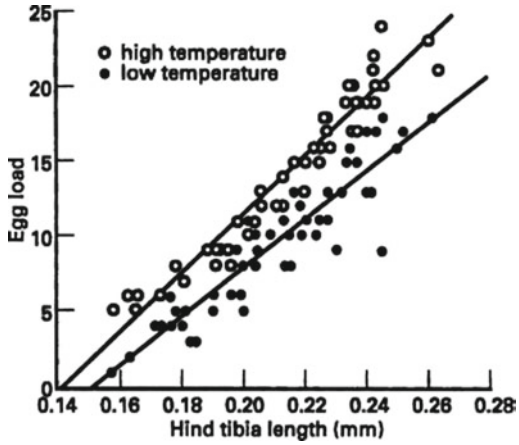


Fig. 2.32 The influence on egg load of parasitoid size and the temperature at which females have previously been held from eclosion, in *Aphytis lingnanensis* (Aphelinidae). *Source* Rosenheim and Rosen (1992). Reproduced by permission of Blackwell Publishing

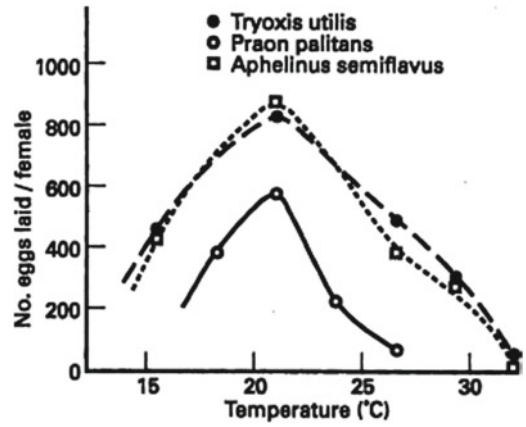


Fig. 2.33 Comparison of mean lifetime fecundity of the aphid parasitoids *Praon palitans*, *Trioxys utilis* (Braconidae) and *Aphelinus semiflavus* (Aphelinidae), over a range of constant temperatures. *Source* Force and Messenger (1964). Reproduced by permission of The Ecological Society of America

of Rosenheim and Rosen's (1992) study are shown in Fig. 2.32, which also shows the influence of body size upon early-life potential fecundity (Sect. 2.7.3).

The effects of climate warming and especially climate extremes are making studies exploring the effects of temperature and other abiotic parameters more and more relevant in the studies of natural enemy-prey/host and multitrophic interactions. The frequency, duration and intensity of climate extremes, such as heat waves, has increased markedly over the past 30 years (Perkins et al., 2012). Because insects and other arthropods are ectotherms, they are potentially exposed to stresses that are becoming unprecedented in their recent evolutionary history (Harvey et al., 2020; Ma et al., 2021). Temperature can alter functional responses in parasitoids and predators and reduce their ability to exploit and suppress their hosts or prey (Romo & Tylianakis, 2013; Kalinkat et al., 2015; Chen et al., 2019a). Over time, this can lead to phenological mismatches between the natural enemies and their prey or hosts (Damien & Tougeron, 2019).

It is generally the case that there is an optimum temperature range outside of which the insect either cannot maintain ovigenesis and oviposition or is unable to do so for long (Force & Messenger, 1964; Greenfield & Karandinos, 1976; Figs. 2.19a and 2.33). Although there is great variation from species to species, the limits to the favourable range for oviposition are often narrower than those for ovigenesis (Bursell, 1964). Within the optimum range, one effect of higher temperature on the pattern of oviposition is to shift the fecundity schedule, with the ovigenesis/oviposition maximum occurring earlier in life (Siddiqui et al., 1973; Ragusa, 1974; Browning & Oatman, 1981; Miura, 1990).

In the coccinellid *Adalia bipunctata* fecundity increases up to 2 °C, correlating well with the increase in food consumption rate. However, above that temperature fecundity declines despite a continued increase in consumption.

Higher temperatures may constrain fecundity through increased metabolic costs i.e., daily maintenance requirement (Mills, 1981; Ellers et al., 2001), although Ives (1981) found no significant influence of temperature on the maintenance requirement of the two *Coccinella* species he studied. More recently, it has been shown that exposure to higher temperatures can induce sterilisation in male parasitoids by killing their sperm, thus preventing their ability to inseminate females (Nguyen et al., 2013). Upper thermal limits on insect survival and fecundity

have been extensively discussed by Bowler and Terblanche (2008) and Walsh et al. (2019).

No attempts appear to have been made to describe mathematically the relationship between oviposition rate and temperature, as has been done with development. Several workers have found that alternating temperatures increase insect fecundity (Messenger, 1964a; Barfield et al., 1977a; Ernsting & Huyer, 1984), and thus it may be invalid to estimate oviposition rates in the field directly from constant temperature data. A similar approach to that used for estimating development based on cyclical temperature regimes might give more meaningful results but has not yet been attempted (Sect. 2.9.3).

Some adult predators may be able to maintain maximal levels of ovigenesis through thermoregulation achieved either by thermal preference behaviour (including basking), by employing physiological mechanisms, and by employing physical adaptations such as melanisation of the integument (Dreisig, 1981; Brakefield, 1985; Miller, 1987; Stewart & Dixon, 1989).

Temperature is known to influence the length of pre-oviposition period in parasitoids and predators (e.g., Stack & Drummond, 1997; Seal et al., 2002). Acclimation to temperature extremes may be useful in inundative releases, but any benefits could be offset by fitness costs (Scott et al., 1997).

2.7.4.2 Light Intensity and Photoperiod

The deleterious effects of light pollution on insects are increasingly being acknowledged (Eisenbeis et al., 2009; Grubisic et al., 2018; Firebaugh & Haynes, 2019). For instance, the intensity, duration and quality of light have an important influence on the biology and behaviour of most insects. High light intensity seems to increase the general activity of diurnal predators and parasitoids. For example, adults of the coccinellid beetle *Cryptolaemus montrouzieri* spend a greater proportion of their time walking and make more attempts to fly in bright light than under dim light conditions (Heidari, 1989). Light quality and intensity may also influence the close-range perception of hosts. Care must therefore be taken in fecundity experiments to provide sufficient light for normal activity, but bear in mind that in the field, bright light conditions are normally associated with increased radiant heat. Laboratory experiments that involve varying light intensity alone will require the radiant heat component of light to be removed, using suitable glass and water filters. Even cold-fibre optic lamps used in microscopy can raise the body temperature of dark-coloured insects by at least 2 °C above ambient. A thermocouple (Unwin & Corbet, 1991) inserted into the body of a dead insect will enable the heat absorbed from a light source to be measured and suitable infra-red filters to be devised (Heidari & Copland, 1993). Owens and Lewis (2018) reviewed studies examining the effects of different kinds of artificial night lights (e.g., incandescent and halogen bulbs) on insects. More studies are needed in order to elucidate the extent to which artificial light disrupts trophic interactions and biological control.

Most natural enemy species will show strong diurnal peaks of behavioural activity, foraging being mainly confined to the photophase, as in many parasitoids and some carabid beetles (Luff, 1978; Ekbom, 1982; Ruberson et al., 1988; Fig. 2.34). The photophase in fecundity experiments should therefore be the same as that experienced in the field; a continuous light regime may result in a higher fecundity than would be achieved in the field (Lum & Flaherty, 1973). Because of its effects on food consumption, photoperiod length may also influence larval growth and development rates in larval predators (which in turn will influence adult fecundity, Sect. 2.7.3) and the rate of ovigenesis.

Weseloh (1986) showed that the egg load of females of the egg parasitoid *Ooencyrtus kuvanae* (Encyrtidae) kept under long-day conditions increases more rapidly than that of females kept under short-day conditions, and that this is reflected in differences in progeny production. *Anagyrus kamali*, an encyrtid parasitoid of the hibiscus mealybug, is unusual in that its lifetime fecundity is highest under conditions of continuous darkness: this life-history characteristic

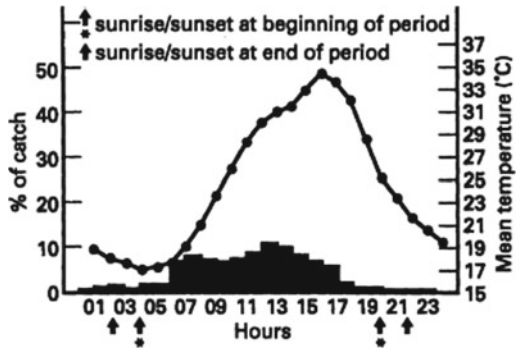


Fig. 2.34 Diurnal flight activity patterns in *Encarsia formosa* (Aphelinidae) in the greenhouse (data for May–June). Percentage of the mean daily catch (by air suction trap) of wasps for each hour (histograms) and mean temperature (curve). *Source* Ekbom (1982). Reproduced by permission of Elsevier Science

would help to keep mass-rearing costs to a minimum (Sagarra et al., 2000b). Hentz et al. (1998) found no significant effect of photoperiod on fecundity in *Chelonus* sp. near *curvimaculatus*.

Photoperiod and light quality and intensity should also be investigated for their effects on reproductive diapause induction (Sect. 2.12.3), particularly where parasitoids and predators are being employed in artifically lit environments (e.g., Stack & Drummond, 1997).

2.7.4.3 Humidity

Decreasing humidity may increase potential and realised fecundity in predators, through an increase in prey consumption by juveniles and females (e.g., Heidari, 1989), but it may also decrease realised fecundity in predators and parasitoids by reducing searching efficiency and longevity (see below). In fecundity experiments care must therefore be taken to control humidity, so that it is around the field average.

2.7.4.4 Field Weather Conditions

The influence of field weather conditions upon the realised fecundity of insect natural enemies has rarely been investigated, undoubtedly because of the often immense practical difficulties involved. Weather can affect fecundity in a variety of ways, through its effects on foraging activity (Fink & Völkl, 1995; Weisser et al., 1997), host/prey and non-host/prey food availability and quality, larval growth rate and survival, ovigenesis, and female survival. Weisser et al. (1997) estimated the lifetime reproductive success (lifetime realised fecundity) of the parasitoid *Aphidius rosae* in relation to wind and rain conditions by means of simulation modelling. They first developed a simulation model to predict patterns of parasitism of aphid colonies in the field as a function of weather conditions, then they parameterised the model using data from both laboratory and field experiments on parasitoids. Periods of relatively 'good' and relatively 'bad' weather were simulated using real weather data. They showed that only a small proportion of females was able to realise oviposition levels close to the maximum lifetime realised fecundity, as measured in the laboratory. Barometric pressure is also likely to affect fecundity in insect natural enemies; Roitberg et al. (1993).

2.8 Adult Longevity

2.8.1 Introduction

The life-span of an individual insect can be divided into two phases: (1) the development period from hatching of the egg until adult eclosion (Sect. 2.9), and (2) the period of adult life, usually referred to as longevity (Blackburn, 1991a, b). An obligatory or facultative period of dormancy may intervene during the lifetime of an individual to extend either development or adult longevity for a variable period of time (Sect. 2.12).

Adult longevity may be studied from a variety of standpoints. For evolutionary biologists, it is a component of individual fitness (Waage & Ng, 1984; Hardy et al., 1992; Roitberg et al., 2001; Rivero & West, 2002; Tylianakis et al., 2004; van Baalen & Hemerik, 2008; Jervis et al., 2008; Snart et al., 2018), the assumption being that: (1) the longer a male can live, the more females he can inseminate, and therefore the more eggs he can fertilise; and (2) the longer a female can live, the more eggs she will lay. In both cases, the

proviso 'all else being equal' applies. Adult longevity is also studied from the point of view of population dynamics, because of its relationship to female fecundity, the prey death rate and the predator rate of increase. Most studies on natural enemies measure adult longevity in the laboratory: there is a dearth of studies that measure it under natural conditions. Individual marking techniques that can be used to measure adult survival in the field are discussed in Chap. 6 (Sects. 6.2.10, and 6.2.11).

Longevity, like fecundity, is a highly variable species characteristic, influenced by a range of physical and biotic factors. The commonest experiments into the effects of these factors involve taking a cohort of standardised females (Sect. 2.7.2) and exposing each of them to one of a range of constant environmental conditions from eclosion until death. Mean length of adult life can be plotted against variables such as body size, temperature, humidity, host or prey density, sugar concentration (in diet), and pesticide or other toxin (e.g., Bt, allelochemical) concentration. However, this method of expressing longevity data has major drawbacks (see below).

Evidence for a reproduction–survival trade-off has been found in some predators and parasitoids in relation to prey availability (Ernsting & Isaaks, 1991; Kaitala, 1991; Kopelman & Chabora, 1992; Valicente & O'Neill, 1995; Ellers et al., 2000; Jervis et al., 2008; Scharf et al., 2013). A cross-species trade-off was also observed in the gerrids that Kaitala (1991) studied. See Dixon (2000) for a discussion of the reproduction-survival trade-off within and among species of Coccinellidae. A cross-species trade-off between ovigeny index and life-span (Sect. 2.3.4) was recorded by Jervis et al. (2001, 2003).

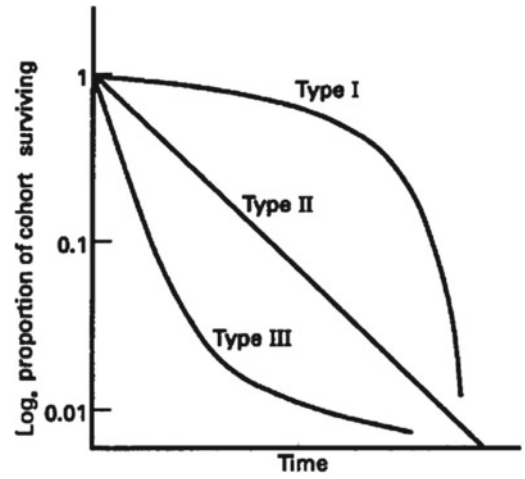


Fig. 2.35 The three main types of survivorship curve: Type I—mortality concentrated in the oldest age classes; Type II—constant risk of death; Type III—mortality concentrated in the youngest age classes. Note the logarithmic scale for the vertical axis

2.8.2 Survival Analysis

Frequently, in the literature, longevity data are presented as the mean length of adult life plus or minus its 95% confidence limit or standard deviation or standard error. However, when statistical comparisons between treatments are made, authors overlook the fact that individual longevity data are rarely normally distributed. For statistical comparisons between treatments to be biologically meaningful, the data are best presented in other ways such as cohort survivorship curves, which show the fraction of each cohort surviving at a particular moment in time (Fig. 2.35). Such curves fall into 3 categories: Type I, in which the risk of death increases with age; Type II, in which there is a constant risk of death, i.e., the risk is independent of age; and Type III, in which the risk of death decreases with age.

Survival data have been compared by plotting survivorship curves and calculating the time to 50% mortality (LT_{50}) for each treatment and assessing the statistical significance of differences in this quantity. A major difficulty with this approach is that, at a particular point on the time axis, one or more of the curves might comprise few observations. Also, the 50% mortality level is subjective. As pointed out by Crawley (1993), generalised linear modelling techniques (available in many statistical software packages) offer one of the best means of analysing survival data. The data can be analysed statistically in terms of

survivorship (proportion of individuals from the cohort still alive at a particular point in time), the age at death, and the instantaneous risk of death (also termed the 'age-specific instantaneous death rate' by biologists or 'hazard rate' by statisticians). Generalised linear modelling can be used to determine which of a variety of available models (exponential, log-normal, Weibull) best describe the observed data. Having decided upon the most appropriate model, the effects of different experimental treatments can then be compared. For details of the procedure, see Crawley (1993, 2002).

The Weibull model has been used to analyse survival data for parasitoids (e.g., Tingle & Copland, 1989; Hardy et al., 1992; Núñez-Campero et al., 2012; Amante et al., 2017; Snart et al., 2018; Jucker et al., 2020). The Weibull frequency distribution was originally considered as a model of human survivorship (Gehan & Siddiqui, 1973) and has commonly been used in engineering as a 'time to failure' model. The Weibull distribution is extremely flexible, possessing either positive or negative skewness, so allowing all three types of survival curve (I, II, III) to be analysed (Cox & Oakes, 1984). The advantage of using the Weibull model to describe survival curves is that it summarises the information contained in a curve as both a rate parameter and a shape parameter. The fraction (F) of the cohort surviving at time t is given by:

$$F = 1 - \exp(-\{(t/b)^c\}) \qquad (2.4)$$

Statistical packages estimate the most appropriate value of the shape parameter c, and allow the rate (or scale) parameter b to be a linear combination of explanatory variables. Hardy et al. (1992) examined survivorship in females of the bethylid wasp *Goniozus nephantidis*, and found that for each of two treatments a curve based on a Weibull distribution showed some systematic deviation from the observed curve. Having noted a relationship between longevity and body size in females (see below), they allowed the logarithm of the distribution's rate parameter to be a linear function of female size. Incorporation of female size significantly improved the fit, and therefore the explanatory power, of the model in the two treatments.

If you are dealing with a particularly long-lived species, you do not have to wait until all the individuals have died in order to terminate an experiment. The experiment can be terminated earlier, and certain statistical analyses can be used to take account of individuals that die at an unknown time (after the end of experimental observations), i.e., insects that are 'censored', statistically speaking (Crawley, 1993, 2002). Such analyses can also be used to take account of individuals that are accidentally lost or killed during the experiment. However, censoring effectively loses information on longevity that it would be better to have and thus offers a means of dealing with a situation that is ideally avoided in the first place. If there are, unavoidably, individuals in the data set with unknown times of death, it is better to use what information is available on their longevity (the time they were known to have lived) by including them as censors than to exclude them from the analysis altogether (the latter approach introduces a bias towards excluding those individuals that live longest).

Siekmann et al. (2001) applied Cox's proportional hazards model (which is available in several statistical software packages) to longevity data obtained for *Cotesia rubecula* given a single meal, the concentration and timing of which varied among treatments. Their analysis showed that the risk of sugar-fed females starving to death was reduced by up to 73% in comparison with unfed wasps, depending on sugar concentration and timing, and that wasps need to locate food at least once a day if they are to avoid starving to death.

2.8.3 Effect of Biotic Factors on Adult Longevity

2.8.3.1 Host and Prey Density

Non-predaceous Females

In some studies, host/prey density appears to have had little or no effect upon adult survival in such insects; at least when average longevity is used as the measure of survival (Mackauer, 1983; Liu, 1985b). Visual inspection of survival curves suggests the same, although a survival analysis of the type discussed above needs to be carried out on such data. Nevertheless, there is evidence for an effect of host availability on survival in some parasitoids. In some species, host-deprived females are able to live longer than undeprived ones kept under otherwise identical conditions (e.g., Tran & Takasu, 2000); presumably they are able to do so because they obtain energy for maintenance from egg resorption, and/or they do not incur the life-span costs of oviposition (see above). A host density-related trade-off between reproduction and adult life-span has been recorded in a few parasitoids. Ellers et al. (2000) exposed *Asobara tabida* to different host density regimes and found that: (1) the total number of eggs produced (those laid in hosts plus those remaining in the females upon death) correlated with host density, and (2) there was a negative linear correlation between physiological life-span and the number of eggs produced—each egg that was produced decreased life-span by an equal amount. The significance of the shape of the trade-off function in *A. tabida* and in insects generally is discussed by Ellers et al. (2000). Another parasitoid species in which there is a host availability-related trade-off between reproduction and life-span is *Leptopilina boulardi* (Kopelman & Chabora, 1992). Apparently in this species life-span declines in relation to increased oviposition rate, given that the species is pro-ovigenic (this rules out an effect of ovigenesis rate) and females in the different host density treatments produced the same number of progeny (see discussion in Jervis et al., 2001).

Predaceous Females

Most information on predaceous females relates to cases where the longevity of females deprived of hosts or prey (deprived for either the whole or part of an experimental period) is compared with that of undeprived females. As one might expect, longevity is found to be shortest in the deprived females: they cannot satisfy their metabolic requirements for maintenance. For instance, in the host-feeding parasitoid *Dicondylus indianus*, female longevity increased with host density (Sahragard et al., 1991). Bellows (1985b) also found that longevity in the host-feeding bruchid parasitoid was greater in wasps provided with mature hosts than wasps with young hosts or no hosts. By contrast, juvenile food limitation extended longevity in the bridge spider, *Larinioides sclopetarius*.

There are few published studies in which the longevity of predaceous females has been related to either availability of prey/hosts or consumption rate. Longevity is positively related to prey consumption rate in ovipositing *Coccinella undecimpunctata* over a wide range of prey densities (Ibrahim, 1955). In nonovipositing *Thanasimus dubius* (Cleridae), longevity becomes a direct function of prey density only at low levels of prey availability (Turnbow et al., 1978); the probable reason for the lack of a relationship at higher levels of prey/host availability in this case is that at these levels the predators' maintenance requirements are fully satisfied. By varying prey availability, Ernsting and Isaaks (1991), Kaitala (1991) and Nakashima and Hirose (1999) obtained evidence for a reproduction–survival trade-off in the predators they studied.

2.8.3.2 Prey and Host Quality

In predatory Coccinellidae and Anthocoridae adult longevity may be significantly affected by the prey species fed upon by the adult (Hodek, 1973; Chyzik et al., 1995; Mendes et al., 2002). This also applies to destructively host-feeding parasitoids (Wilbert & Lauenstein, 1974). The host stage fed upon influences longevity in the

host-feeding bethylid *Cephalonomia stephanoderis* (Lauzière et al., 2000). With both host/prey species and host/prey stage effects, it is important to establish whether they are attributable to differences in the quantity of prey/host materials ingested or differences in host/prey quality sensu stricto. In parasitoids, host species affects longevity via its effect on body size (see above).

2.8.3.3 Host-feeding by Parasitoids

Consumption of host haemolymph improves longevity in some host-feeding wasp species (e.g., *Eupelmus vuilletti:* Giron et al., 2002; *Neochrysocharis formosa*: Liu et al., 2015) but not in others (e.g., *Diadromus subtilicornis*: Tran & Takasu, 2000; *Gelis agilis:* Harvey, 2008), while in the host-feeding parasitoid wasp *Aphytis melinus*, consumption of host blood positively influences longevity only if sugar-rich food is also taken (Heimpel et al., 1997a) (see Jervis & Kidd, 1986, and Heimpel & Collier, 1996, for reviews). Kapranas and Luck (2008) found that host-feeding had differing effects in two congeneric parasitoids of scale insects. In *Metaphycus flavus*, resources obtained during host-feeding were used primarily for egg production, whereas resources obtained by *M. luteolus* during host-feeding were used also for maintenance. By directly injecting females with the sugars that are abundant in host blood (trehalose, sucrose), Giron et al. (2002) showed that these sugars are solely responsible for the greater longevity of host-fed females.

2.8.3.4 Non-host and Non-prey Foods

Many studies have shown that, in the absence of hosts or prey, many parasitoids and predators given carbohydrate-rich foods, e.g., diluted honey solutions, live significantly longer than insects that are either starved or given only water (see reviews by Hagen, 1986; Jervis & Kidd, 1986; van Lenteren et al., 1987; Heimpel & Jervis, 2004; Jervis et al., 2008; see also Chap. 8).

Several studies have also revealed that longevity varies with the quality of food consumed. A simple experiment involves providing predators or parasitoids with one of a range of different diets, e.g., different sugars or combinations of sugars in solution, or even different nectars or honeydews, and comparing the effects of these on survival. However, for investigations of the effects of non-host food consumption on longevity (and fecundity) to have relevance to the field situation (particularly in biological control), they should involve first identifying the natural diet of parasitoids and predators, and then providing insects with the same or very similar foods (Chap. 8).

For details of protocols for determining the effects of biochemical components of non-host foods on longevity, see Finch and Coaker (1969) and Wäckers (2001). The effects of sugar-feeding on carbohydrate and lipid levels in parasitoids have been investigated by Olson et al. (2000), Fadamiro and Heimpel (2001) and Casas et al. (2003) (see Sect. 2.13 for biochemical techniques).

2.8.3.5 Body Size

A positive correlation between body size and longevity, at least across the lower range of host body sizes, has been shown for the adults (in some cases males as well as females) of several parasitoid species (e.g., Sandlan, 1979; Mani & Nagarkatti, 1983; Waage & Ng, 1984; Bellows, 1985b; Hooker et al., 1987; van den Assem et al., 1989; Hohmann et al., 1989; Heinz & Parrella, 1990; Hardy et al., 1992; Harvey et al., 1994; West et al., 1996; Ellers et al., 1998; Fidgen et al., 2000; Rivero & West, 2002; reviewed by Visser, 1994). Exceptions include *Goniozus nephantidis* (in which larger females live longer than smaller ones if hosts are provided, but smaller females live slightly longer than larger ones if hosts are not available; Hardy et al., 1992), and both *Asobara tabida* and *Nasonia vitripennis* (in which the body size effect does not occur in fed females; Ellers et al., 1998; Rivero & West, 2002). The size effect upon the longevity of unfed female *A. tabida* and *N. vitripennis* is attributable to the smaller fat reserves of small females: such females can obtain additional energy for maintenance by feeding, but note that females cannot either supplement or replenish their fat body reserves (Ellers et al., 1998; Rivero & West, 2002; see also Sect. 2.14). Interestingly, in the two *Metaphycus* species

studied by Bernal et al. (1999) the body size effect occurs in fed females and not in unfed ones; the amounts of lipid reserves were not measured in this case.

Because a size–longevity relationship may not appear to exist for some species (e.g., Takagi, 1985, on *Pteromalus puparum*), it is important, in experiments, to provide parasitoids with a range of host sizes equivalent to that occurring in the field. Few studies have measured longevity in relation to body size under actual field conditions (West et al., 1996).

Blackburn (1991a) showed through a comparative analysis (Sect. 1.2.3) of 474 hymenopteran parasitoid species that, across the species within a taxon, there is no correlation between body size and life-span (see Blackburn, 1991a, and Jervis et al., 2003, for discussion). Sokolovska et al. (2000) found positive correlations between longevity and male and female body size among Odonata, but their meta-analysis has been criticised by Thompson and Fincke (2002).

Burkhard et al. (2002), used the degree of wing wear and injury to estimate size-specific survivorship in field populations of the predatory fly *Scathophaga stercoraria*, and showed that in females longevity increased with body size in both flight seasons but that in males it increased slightly in the spring and decreased in the autumn. Burkhard et al. (2002), however, urge caution in applying the method (see their paper for details).

2.8.3.6 Mating

As discussed in Chap. 4 (Sect. 4.5.3), frequent mating may shorten life-span in both females and males. Several laboratory studies have shown that in predatory coccinellids unmated females live longer than mated ones; the same also applies to males (Dixon, 2000; see also Taylor et al., 1998, on a predatory stonefly). However, there is no difference in longevity between mated and virgin females of the predatory bug *Podisus maculiventris* (De Clercq & Degheele, 1997).

In experiments aimed at testing for the effects of mating on longevity, one must establish that mating really has occurred (see 'mating' in Sect. 2.7.3). If a positive effect of mating upon female longevity is found, the question arises as to whether longevity is enhanced due to the nutrient contribution made by the male, in the form of sperm or spermatophore.

2.8.4 Effect of Physical Factors on Adult Longevity

2.8.4.1 Temperature

There will be an optimum range of temperatures outside of which survival is severely reduced (Jackson, 1986; Krishnamoorthy, 1989; Mohan et al., 2004). In general, and usually in males as well as females, longevity decreases with increasing temperature within the optimum range (Sahad, 1982, 1984; Nealis & Fraser, 1988; McDougall & Mills, 1997; Hentz et al., 1998; Tran & Takasu, 2000; Liu & Tsai, 2002; Seal et al., 2002; Emana, 2007; Dhillon & Sharma, 2009; e.g., Fig. 2.36), although for some species no more than a trend may be apparent (Barfield et al., 1977a; Cave & Gaylor, 1989; Miura, 1990).

Most experiments designed to demonstrate the effect of temperature on adult longevity involve exposing insects to constant temperatures, which ignores the fact that in nature temperatures will fluctuate during each day, the lowest temperatures occurring at night. Ideally, longevity ought to be studied at temperature extremes that are

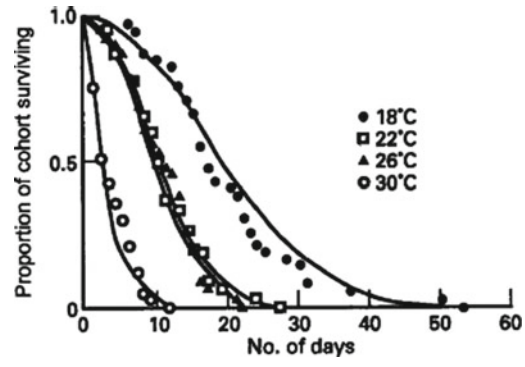


Fig. 2.36 Survivorship of *Anagyrus pseudococci* (Encyrtidae) at four different constant temperature regimes. *Source* Tingle and Copland (1989). Reproduced by permission of Lavoisier Abonnements

part of a cyclical regime, but such an approach has rarely been adopted. Ernsting and Isaaks (1988) measured the survival of the carabid *Notiophilus biguttatus*, given excess prey, at a constant 10 °C regime compared with a daily fluctuating (20 °C day/10 °C night) regime. The lower survival of beetles held under the fluctuating regime could simply be explained by the higher average daily temperature at that regime. Minkenberg (1989) incorporated a more realistic fluctuating temperature regime in his experimental design. He exposed the eulophid *Diglyphus isaea* to each of three constant temperatures (15°, 20°, 25 °C) and to a fluctuating regime that involved the temperature increasing linearly from 0100 to 0300 h, decreasing from 1500 to 1700 h, and being fixed at 22 °C from 0300 to 1500 h and at 18 °C from 1700 to 0100 h. Survival of wasps held under the fluctuating regime (average daily temperature 20.3 °C) was much lower than at the constant 20 °C regime.

Some parasitoids and predators overwinter as adults and may be exposed to near- and/or subzero temperatures. Cold tolerance of adult *Bathyplectes curculionis* (Ichneumonidae) was studied by Berberet et al. (2002), and that of *Harmonia axyridis* (Coccinellidae) by Watanabe (2002).

2.8.4.2 Humidity

It is clear from many experimental studies that natural enemy adults have particular humidity requirements for survival (Kfir, 1981; Hérard et al., 1988; Wysoki et al., 1988; Emana, 2007). Although it would appear to be quite easy to carry out an experiment designed to measure survival at different humidities, there is the problem of maintaining the insects for a sufficiently long period for statistical comparisons to be made. Insects deprived of food are likely to die quite quickly, but if they are provided with honey or sucrose solutions (see above), it may be difficult to separate the effects upon longevity of the water content of the air and that of the food. Similarly, it may be difficult to set up an experiment that incorporates some degree of biological realism in the form of a plant surface, since the latter will be actively transpiring.

Small-bodied insects, because of their high surface area to volume ratio, will be more prone to desiccation at low humidities than large-bodied insects; see Jervis et al. (2003) who discuss this, from a comparative perspective, in relation to parasitoid wasps.

2.8.4.3 Photoperiod

Little is known about the influence of photoperiod on longevity. Given that some predaceous insects are active only during certain periods of the day or night, one might expect longevity to be influenced by photoperiod. In the parasitoid *Ooencyrtus kuvanae,* photoperiod experienced upon adult eclosion influences both longevity and the rate of progeny production. Short-day conditions resulted in females producing fewer progeny but living longer. Switching photoperiods after twelve days failed to alter this once it had been established (Weseloh, 1986). In *Anagyrus kamali*, longevity, like lifetime fecundity, is highest under continuous darkness (Sagarra et al., 2000a). Hentz et al. (1998), however, found no significant effect of photoperiod on longevity in *Chelonus* sp. near *curvimaculatus*. Note that some entomophagous insects are nocturnal, e.g., certain Ichneumonidae (Gauld & Huddleston, 1976), Vespidae, Pompilidae and Rhopalosomatidae (Gauld & Bolton, 1988).

2.9 Growth and Development of Immatures

2.9.1 Introduction

Development refers to the morphological, anatomical and physiological changes shown by each individual insect from the time the egg is laid to the time the adult ecloses. Growth refers to the increase in biomass of the insect during the period between hatching from the egg and the end of the larval phase of the life-cycle (Fig. 2.37) or between instars. The larval phase in predators comprises long periods of feeding and brief periods of moulting. Typically, biomass increases steadily throughout each instar. At the time of the moult, biomass falls slightly due to

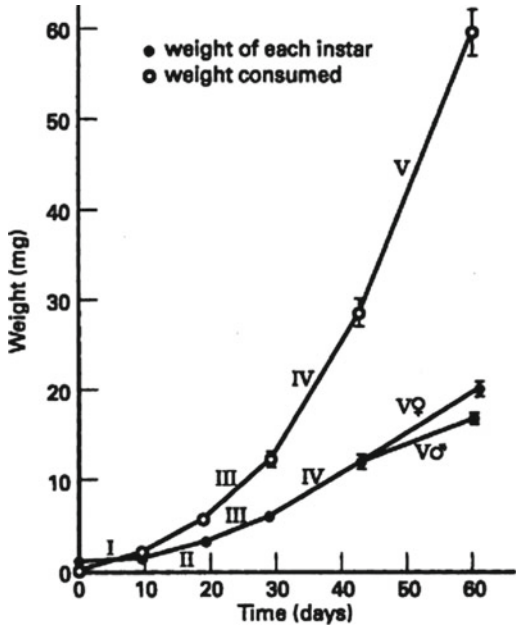


Fig. 2.37 Development in the bug *Blepharidopterus angulatus* (Miridae) given excess prey, showing the length of time spent in each larval instar, the body weight at the start of each instar, and the cumulative wet weight of lime aphids consumed up to the start of each instar. Roman numerals denote instars. Points are shown ± SE. *Source* Glen (1973)

the loss both of the exuvium and of some water (which is not immediately replaced, as the insect is not feeding) (Chapman, 1998, 2013). In some aquatic insects there is no decrease in biomass at the moult; instead there is an increase due to absorption of water through either the cuticle or the gut; in *Notonecta glauca* this increase is very large (Wigglesworth, 1972).

In the field, as opposed to laboratory, measurements of growth and development in predators and parasitoids have been made on few species (e.g., Griffiths, 1980, on ant-lions; Banks & Thompson, 1987b, on damselflies).

For predators the protocols for measuring growth and development in relation to certain physical and biotic factors are relatively straightforward. For example, to study the influence of prey availability, take a series of cohorts of newly hatched larvae and present each insect with one of a range of chosen prey densities (prey of a fixed size), at a constant temperature, humidity, photoperiod, either for the duration of the insect's life or for the duration of one or a few instars only. On each day of larval life, replace the prey. Larval development is measured simply in terms of the period of time between moults or other events (e.g., egg hatch and pupation). Larval growth can be measured as the dry or fresh weight gain, including exuvia weight, or the body size increase (e.g., measured in terms of head width) between instars, although the standard measure of growth rate is the mean relative growth rate (MRGR):

$$\text{MRGR} = \frac{\ln(W_f) - \ln(W_i)}{d} \qquad (2.5)$$

where W_i is the initial weight of the insect, W_f is the final weight of the insect, and d is the period of time over which growth is measured. Some workers (Paradise & Stamp, 1990, 1991) have expressed growth rate differently, as the fresh weight gained/instar duration × the average fresh weight of the predator during the instar.

For parasitoids, the protocols for measuring the influence of physical and biotic factors on growth and development may be rather more complex than for predators. Endoparasitoids are a particular problem, since the sizes and weights of larvae cannot easily be measured and the larvae often cannot easily be assessed as to their stage of development (Mackauer, 1986; Sequeira & Mackauer, 1992b; Harvey et al., 1994). However, for ectoparasitoids and predatory arthropods, it is generally much easier to measure the growth and development of immature stages to adult (Harvey et al., 1998; Dmitriew and Rowe, 2003; Jespersen & Toft, 2003; Singh & Mishra, 2014; Harvey, 2021; see below).

A necessary prerequisite for studying many aspects of larval development, particularly instar-related aspects of biology, in predators and parasitoids is the ability to distinguish between the different instars. In some cases, it is relatively easy to tell the instars apart, using features such as mouthpart structure, head capsule width, the degree of wing development, the number and position of prominent setae, spines and other cuticular structures, the structure of the tracheal

system and associated spiracles, and body colour patterns. However, in other insects, obvious distinguishing features may be lacking. Morphometric techniques may therefore be required. Thompson (1975, 1978), for example, decided upon the instar of the damselflies larvae he studied (*Ischnura elegans*), by means of both a frequency distribution plot of head widths of randomly field-collected larvae and a regression of modal head width against probable instar number. Even better discrimination between instars was obtained by plotting head width against body length (Thompson, 1978).

2.9.2 Effects of Biotic Factors on Growth and Development

2.9.2.1 Food Consumption

Introduction

Predator larvae need to consume several prey individuals during development, and each successive instar will show a maximum rate of growth and development at different levels of prey availability. Generally, with increasing prey density, at least across the low and medium ranges, larval predators consume more prey, develop faster, gain more weight and so attain a higher final size (Dixon, 1959, 2000; Lawton et al., 1980; Scott & Barlow, 1984; Pickup & Thompson, 1990; Zheng et al., 1993a, b; Bommarco, 1998; Dmitriew & Rowe, 2007; Harvey, 2021). Where development rate increases non-linearly with prey consumption rate (see below), development rate stops increasing above a certain prey density while growth continues. Growth and development also vary in relation to prey quality.

Food consumption by insects is a subject in its own right, and the associated literature is very large (Waldbauer, 1968; Beddington et al., 1976; Kogan & Parra, 1981; Scriber & Slansky, 1981; Slansky & Scriber, 1982, 1985; Slansky & Rodriguez, 1987; Farrar et al., 1989; Karowe & Martin, 1989). The approach we are recommending here is that of Beddington et al. (1976), as it provides one of the most useful bases for predicting predator–prey population dynamics (Chap. 7). The various problems inherent in measuring food consumption and utilisation by insects and other arthropods are discussed in Waldbauer (1968), Lawton (1970), Ferran et al. (1981) and Pollard (1988).

The basic protocol for studying the effects of prey availability and prey consumption on growth and development in predators has already been outlined. Other measurements can also be taken in order that various nutritional indices can be calculated; these measurements are of:

1. The biomass of the prey materials ingested (biomass is best measured in terms of dry weight, since prey remains are likely to lose water before retrieval). The predator's efficiency of conversion of ingested food into body substance (ECI) can then be calculated as follows:

$$\text{Conversion efficiency} = \frac{M}{C - D} \times 100 \tag{2.6}$$

where M is the increase in biomass of the predator, C is the biomass of captured prey, and D is the biomass of the captured prey that is not consumed (C–D is therefore the biomass of prey actually ingested). According to Cohen (1984, 1989), predaceous insects with piercing, suctorial mouthparts (e.g., Heteroptera) ought to have higher ECI values than predators with chewing mouthparts, because they obtain a larger proportion of highly digestible materials from their prey (a process assisted by pre-oral digestion) (see also Cohen, 1995, and Cohen & Tang, 1997). The ECI is a measure of gross growth efficiency, since biomass losses in the form of faeces and excreta are not accounted for.
2. C, D and the biomass which appears as faeces (F), and the products of nitrogenous excretion (U). The predator's utilisation efficiency, i.e., the efficiency with which the prey biomass captured is converted into predator biomass, can then be calculated:

$$\text{Utilisation efficiency} = \frac{C - D - F - U}{C} \times 100 \tag{2.7}$$

3. C, D, F and U (as in 1. and 2.). The predator's assimilation efficiency, i.e., the efficiency with which the prey biomass consumed is converted into predator biomass, can then be calculated:

$$\text{Assimilation efficiency} = \frac{C - D - F - U}{C - D} \times 100 \tag{2.8}$$

4. C, D, and F (as in 1. and 2.). The predator's digestive efficiency (also termed 'approximate digestibility'), i.e., the efficiency with which the prey biomass ingested is digested and absorbed, can be calculated:

$$\text{Digestive efficiency} = \frac{C - D - F}{C - D} \times 100 \tag{2.9}$$

Other nutritional indices used in studies of food consumption by insects are discussed by Waldbauer (1968) and Slansky and Scriber (1982, 1985).

Growth Rate

At least some of the food a larva consumes needs to be allocated to maintenance metabolism. Because of this, growth will stop if consumption falls below a certain threshold (this threshold will become higher as the insect grows and its maintenance requirements increase). The energy allocated to growth can be assumed to be a linear function of food intake (Beddington et al., 1976):

$$G = \delta(I - B) \tag{2.10}$$

where G is the growth rate (biomass accumulated per unit time, e.g., fresh weight gain, including exuvium weight, divided by the number of days spent in the instar) of each juvenile stage, I is the rate of ingestion of food (biomass of prey consumed per unit time, in comparable units to G, see Eq. 2.6), and δ and B (the threshold ingestion rate, analogous with parameter c in Eq. 2.1) are constants. Mills (1981) gives an alternative model.

Figure 2.38 shows the relationship between growth rate and consumption rate in larval *Notonecta*; the relationship conforms to that predicted by Eq. 2.10. As can be seen from the intercept of the line with the x-axis, the predator needs to consume a minimum amount of food for any growth to occur.

Should the increase in respiratory rate be non-linear, then growth rate will be non-linear and conform to the following model (Beddington et al., 1976):

$$G = \delta(\log_e I - B) \tag{2.11}$$

Development Rate

If W_i is the initial weight (biomass) of an instar (teneral weight), W_f is the final weight achieved, and W is the total weight gain, then $W = W_f - W_i$. The ratio W/G will define the duration, d, of the instar, and development rate, $1/d$, is given by the following linear model (Beddington et al., 1976):

$$\frac{1}{d} = \frac{\delta}{W}(I - B) \tag{2.12}$$

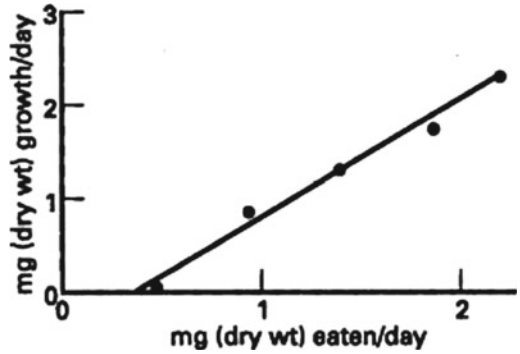


Fig. 2.38 Growth rate as a function of ingestion rate in final instar of *Notonecta undulata*. *Source* Beddington et al. (1976), who used data from Toth and Chew (1972). Reproduced by permission of Blackwell Publishing

If it is assumed, for simplicity's sake, that W remains a constant, then (Beddington et al., 1976):

$$\frac{1}{d} = \alpha(I - B) \qquad (2.13)$$

where α and B are constants. Equation 2.13 still predicts a simple, linear relationship between development rate and consumption rate.

As pointed out by Beddington et al. (1976), Eq. 2.12 ignores the fact that the larvae of some predators may, under conditions of food scarcity, moult to the next instar at significantly lower body weights than when food is abundant. W_i, W_f and W are therefore functions of consumption rate and thus of prey availability: weight gain in each instar cannot be assumed to be constant. Figure 2.39 shows how, in the damselfly *Ischnura elegans*, larvae fed at low prey densities moulted to smaller individuals, i.e., they moulted earlier than better fed larvae, having gained less weight. Mills (1981) demonstrated, through a regression analysis of the relationship between W and consumption rate and teneral weight in *Adalia bipunctata*, a significant dependence in both cases; consumption rate explained 47–75% of the variance.

Thus, the relationship in some predators is more complex than that described by Eq. 2.13. Lawton et al. (1980) provide the following non-linear model:

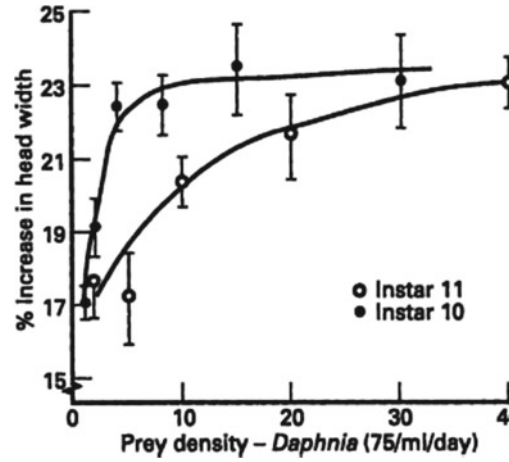


Fig. 2.39 The effect of prey density on the percentage increase in head width at the moult in *Ischnura elegans* (vertical bars = ± SE). *Source* Lawton et al. (1980). Reproduced by permission of Blackwell Publishing

$$\frac{1}{d} = \alpha(\log_e N_a - B) \qquad (2.14)$$

where N_a is the number of hosts fed upon. An alternative non-linear model is provided by Mills (1981). Both models describe a decelerating curve for the relationship between development rate and consumption rate. Curves of this type were obtained in the laboratory for both *Ischnura elegans* (Fig. 2.40) and *Adalia bipunctata* (Fig. 2.41) Lawton et al. (1980) gave, as well as

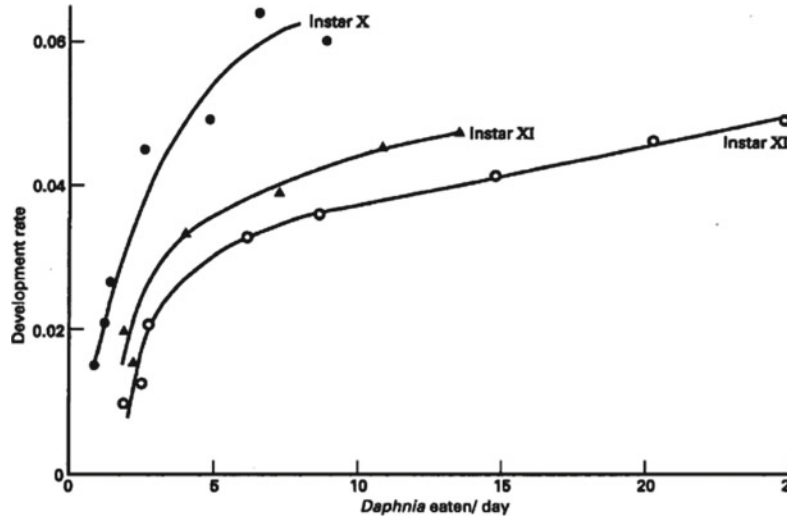


Fig. 2.40 Development rates in *Ischnura elegans* (Odonata: Zygoptera) larvae as a function of the number of prey killed per day. Only larvae that successfully completed their development in each stage were included in the calculations. *Source* Lawton et al. (1980). Reproduced by permission of Blackwell Publishing

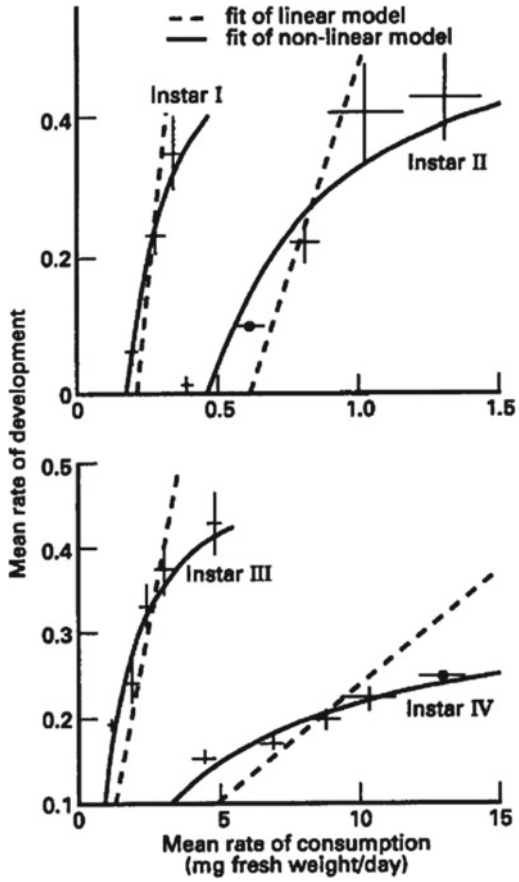


Fig. 2.41 The relationship between mean development rate and consumption rate for the four larval instars of *Adalia bipunctata* (Coccinellidae). Indicated is the fit of linear and non-linear models of development (see text). *Source* Mills (1981). Reproduced by permission of Blackwell Publishing

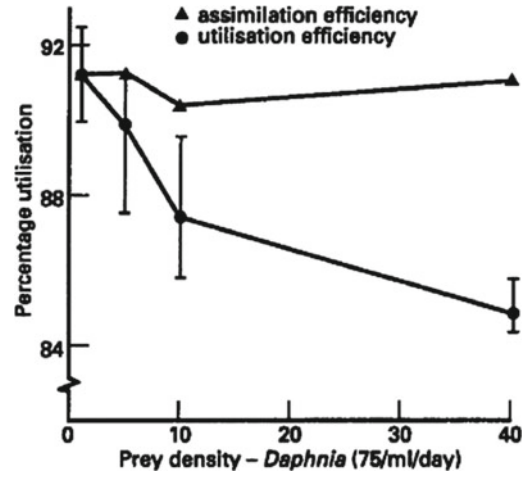


Fig. 2.42 The relationships between assimilation and utilisation efficiencies and prey density in the eleventh instar of *Ischnura elegans* (Odonata: Zygoptera). Utilisation efficiency clearly declines with increased prey density. *Source* Lawton et al. (1980). Reproduced by permission of Blackwell Publishing

a dependence of W on consumption rate, three other reasons to account for non-linearity in the case of *Ischnura*:

1. Variation in k (Eq. 2.2) with prey availability. In *Ischnura* k declined with prey availability (Fig. 2.42), the predators wasting proportionately more of each of the prey they kill at higher densities (adaptive behaviour in many predators, Sect. 1.14; Cook & Cockrell, 1978; Giller, 1980; Sih, 1980; Kruse, 1983; Bailey, 1986; Dudgeon, 1990, although some predators may go to the extreme of not consuming any part of the prey, Yasuda, 1995). However, as Lawton et al. (1980) point out, a decline in utilisation efficiency in *Ischnura* cannot be the sole reason for the non-linear dependence of development rate upon prey consumption rate. If it is, daily growth rates plotted against prey biomass assimilated (C–$[D + F]$) ought to be linear (Eq. 2.10): they are not (Fig. 2.43).
2. A decrease in assimilation efficiency with increasing consumption rate. Lawton (1970) had suggested that this can occur with overfeeding at high levels of prey availability, causing defaecation to take place before digestion is complete. Lawton et al. (1980) investigated whether assimilation efficiency varied with prey availability. Since it does not do so in *Ischnura* (Fig. 2.42), this hypothesised effect could not account for the non-linear dependence of development rate on consumption rate.
3. A non-linear increase in respiratory rates with increasing consumption rate (Eq. 2.11). Lawton et al. (1980) concluded that this effect, together with the variation in k and W, accounted for the observed relationship in Fig. 2.40. Circumstantial evidence to support the conclusion regarding change in respiratory

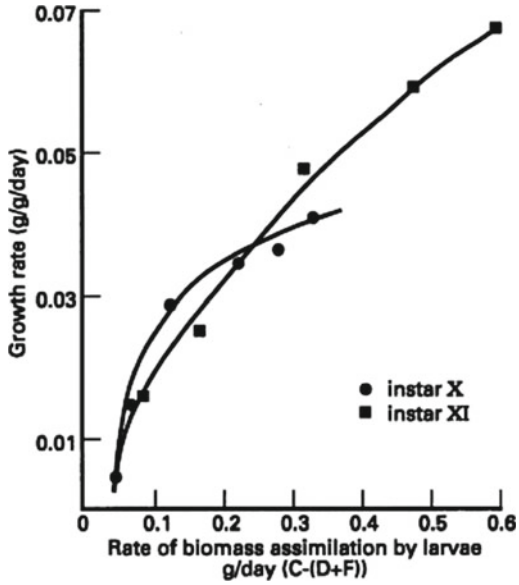


Fig. 2.43 The effect of daily rate of biomass assimilation on growth rate for instars X and XI of *Ischnura elegans* (Odonata: Zygoptera). Growth rate is measured as g/g/d increase in weight and is calculated by dividing weight gained during the instar-by-instar duration. These figures were corrected for the initial weight of the larvae. Wet weights were used for initial and final weights. Only larvae that successfuly completed their development in each instar were used in the calculations. *Source* Lawton et al. (1980). Reproduced by permission of Blackwell Publishing

rates comes from Lawton et al.'s (1980) behavioural observations: larvae held at high prey densities frequently engage in more waving of the gills than other larvae, suggesting that they are under oxygen stress. Respirometric methods would need to be employed to establish whether respiratory rates do indeed alter.

To obtain the relationship between development rate and prey availability, both Eq. (2.13) and Eq. (2.2) can be incorporated into the simple functional response model (Holling's (1966) disc equation; Sect. 1.14) (Beddington et al., 1976):

$$\frac{1}{d} = \alpha\left(\frac{ka'NT}{1 + a'T_hN} - B\right) \qquad (2.15)$$

Equation 2.15 describes a decelerating curve, like a Type 2 functional response (Sect. 1.14). As pointed out by Beddington et al. (1976), the curve is unlikely to go through the origin. Unless the weight at which a species is able to moult to the next instar is very flexible, the effect of B will be to displace the curve along the prey axis. Put another way, there will be a threshold prey density (and therefore consumption rate) below which growth and development cannot take place. Examples of this are shown in Fig. 2.44. In those species that consume proportionately less of each prey item when encounter rates, i.e., levels of prey availability, are high (k declines) the curve will be somewhat different in shape: flatter-topped, with an earlier 'turnover' point (Beddington et al., 1976; also see Yasuda, 1995).

2.9.2.2 Variation in Growth and Development Between and Within Instars

Figure 2.37 shows both the cumulative increase in prey biomass consumed and the increase in weight of nymphs of the bug *Blepharidopterus angulatus* as they develop. Later instars account for most of the total consumption and growth that occurs. In the green lacewing *Chrysoperla carnea* the third (final) instar accounts for 80.5–82.8% of the total consumption and 80.45–85.6% of the total growth that occurs (data from Zheng et al., 1993a).

Figure 2.44 shows that the development rate *versus* prey availability curves differ between instars. As pointed out by Beddington et al. (1976), this is to be expected from the between-instar differences that exist with respect to: (1) attack rate (a') and handling time (T_h), both of which are parameters in the functional response model); (2) metabolic rate, which will increase with instar by a certain power of the body weight —this affects B in Eq. (2.14); and (3) the constants α and k (Beddington et al., 1976).

Examination of the growth rate *versus* consumption rate plots for *Adalia bipunctata* (Fig. 2.45) reveals that the slope (which represents

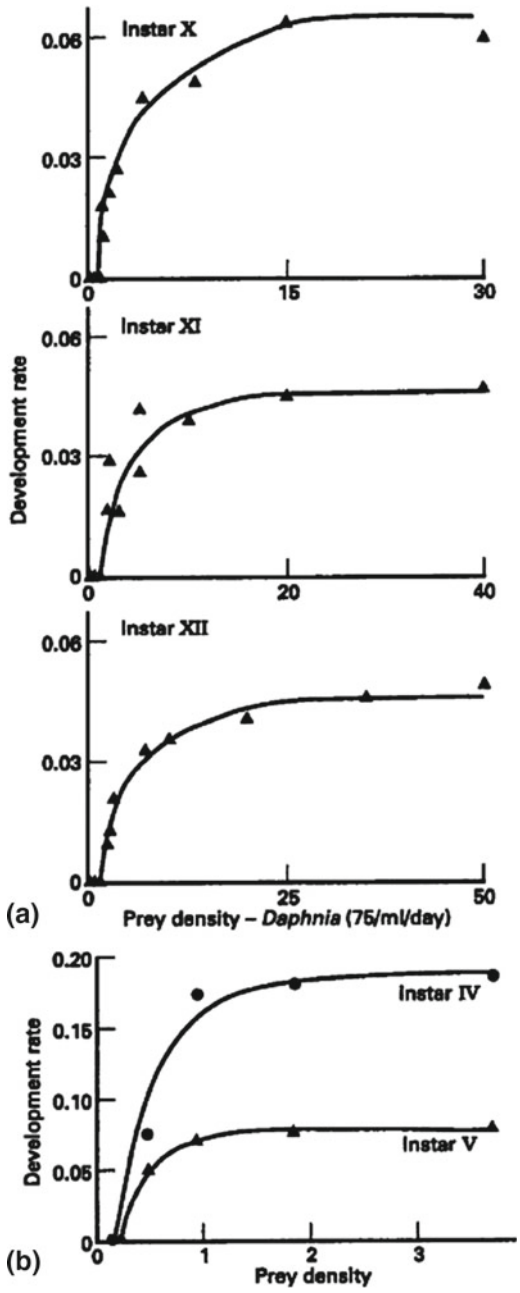


Fig. 2.44 Development rates as a function of prey density in different instars of: **a** *Ischnura elegans* (Odonata: Zygoptera) (*source* Lawton et al., 1980); **b** *Notonecta undulata*. *Source* Beddington et al., 1976, who used data from Toth & Chew, 1972. Reproduced by permission of Blackwell Publishing

conversion efficiency) decreases as the insects pass through the instars. This change in the slope is partly attributable to increased metabolic costs in later instars, as can be seen from the intercepts with the y-axis, representing basal respiratory rates. However, the main cause is likely to be a decline in digestive efficiency, since compared with earlier instars, later instars of *Adalia* consume a greater proportion of each prey item, i.e., *k* increases with instar (Mills, 1981). To understand the relationship between the proportion of each prey consumed and digestive efficiency, consider the surface area/volume ratio difference between food boluses of different sizes. A larger bolus will have proportionately less of its surface area exposed to digestive fluids than a smaller bolus.

Conversion efficiency can also vary with consumption rate within an instar. Third-instar larvae of *Chrysoperla carnea* provided with low prey densities have, as expected, a reduced consumption rate compared with third-instar larvae given high prey densities, but they have a higher conversion efficiency (Zheng et al., 1993a). A similar difference in conversion efficiency is shown by the early instars of the bug *Blepharidopterus angulatus* (Glen, 1973). Two possible reasons for this effect in the case of *C. carnea* were put forward by Zheng et al. (1993a): (1) digestive efficiency is increased, due to the smaller quantities of prey being ingested by larvae given low prey densities; (2) third-instar larvae, like some spiders, reduce their metabolism in response to prey scarcity.

2.9.2.3 Feeding History

Can predators recover from the deleterious effects upon growth and development brought about in previous instars by prey scarcity? To answer this question, a cohort of larvae can be exposed to high levels of prey availability throughout two instars, e.g., the third and the fourth in a coccinellid, and another cohort can be exposed to a prey availability regime that alters from low to high between these two instars. The

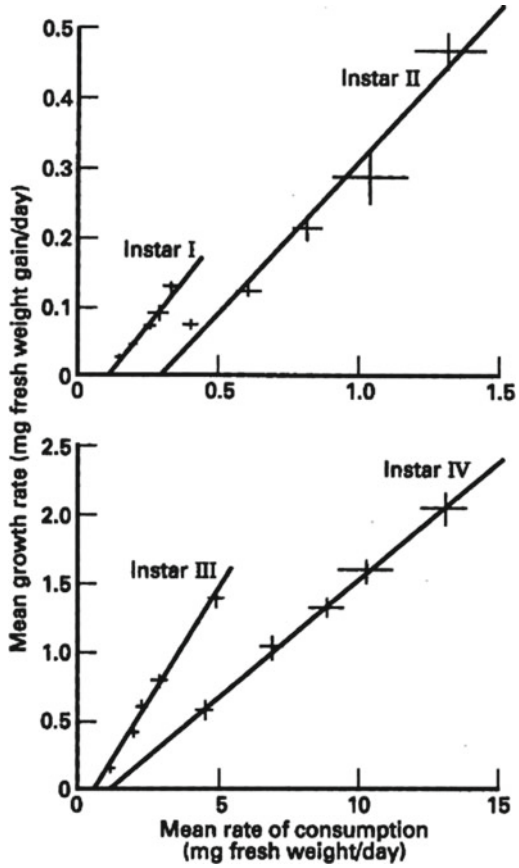


Fig. 2.45 The linear dependence of average (± SE, n = 6–10) growth rates on food consumption rate for the four larval instars of the coccinellid beetle *Adalia bipunctata*. Note that the slope of the relationship, representing the gross food conversion efficiency, decreases as the insect develops. This is partly due to increased metabolic costs, as can be observed from the y-axis intercepts representing basal respiratory rates, but it is mainly due to a decline in digestive efficiency with instar. *Source* Mills (1981). Reproduced by permission of Blackwell Publishing

fourth-instar insects from the two regimes can then be compared with respect to weight gain and instar duration. In this experiment, consumption rate and the various nutritional efficiencies should be measured to determine whether the compensatory effects shown by the test cohort are a result of changes in one or more of these factors within the later instar.

A similar experiment to the above was carried out by Paradise and Stamp (1991) on the mantid *Tenodera sinensis*. These authors showed that: (1) first- and second-instar mantids given a small quantity of prey attained a smaller size and spent more time in those instars than mantids provided with as much prey as they could eat, but that (2) in two out of three cohorts, mantids reared during the first instar on a poor diet recovered during the second instar when they were switched to a higher diet, gaining as much weight as, and spending less time in that instar than, those given a high diet throughout. The larvae of the later instar compensated for poor feeding in the earlier instar by having a higher consumption rate.

Zheng et al. (1993a) conducted a similar experiment with the green lacewing, *Chrysoperla carnea*, but over the entire larval development period. Larvae were either provided with a large quantity of prey over all three instars (HHH regime), or they were given a low quantity over the first two instars and a large quantity during the third (LLH regime). No significant difference in the duration of the third instar was found between larvae in the two regimes, but the overall duration of development from eclosion to pupation was significantly longer in the LLH larvae, i.e., recovery in development rate was partial. The dry weight gain of third-instar larvae was not significantly different in the two treatments, and the same applied to the overall weight gain over the whole of larval development, i.e., recovery in growth was complete. Third-instar larvae in the LLH regime consumed as many prey as those in the HHH regime, and the same applied to larvae over the whole of their development.

Limited recovery from suboptimal feeding conditions can, at least in the laboratory, be achieved in some Odonata (*Lestes sponsa*) by the larva passing through an additional instar. However, instar number is constrained and an increase in any linear dimension is limited to around 25–30% (D.J. Thompson, personal communication).

Can predators with higher growth rates in one instar maintain the advantage through subsequent

instars? To answer this question, the aforementioned experimental design can be reversed, so that in the test cohort the prey availability regime alters from high to low. Experiments carried out by Fox and Murdoch (1978) on the backswimmer *Notonecta hoffmani* show that larvae can maintain a growth advantage during larval development.

2.9.2.4 Non-prey Foods

As with the fecundity *versus* prey density relationship, two effects of providing non-prey foods together with prey might be to lower the prey ingestion rate threshold, thus shifting the development rate *versus* prey availability curve nearer to the origin, and to alter the shape of the curve. Predator larvae may require a lower minimum number of prey items in order to develop at all, and they may develop more rapidly at and above this minimum.

That development rate is increased by provision of non-prey foods is demonstrated by experiments conducted on larvae of the lacewing *Chrysoperla carnea* (McEwen et al., 1993). At the three test prey densities offered to the predators during development, larvae given an artificial honeydew with prey required significantly fewer prey, developed significantly more rapidly, and attained a significantly higher adult weight than larvae given water with prey. Some predators can complete larval development when prey are absent, if certain non-prey foods are available, e.g., the bug *Orius insidiosus* (Anthocoridae) (Kiman & Yeargan, 1985), and the coccinellid *Coleomegilla maculata* (Smith, 1961, 1965). Predators such as the bug *Blepharidopterus angulatus* cannot complete development on a diet of honeydew alone, but nymphs that are switched from honeydew to a diet of aphids after the third instar can complete development (Glen, 1973).

2.9.2.5 Prey Species

Larval growth and development might be expected to vary in relation to prey species. Examples of studies demonstrating this effect in coccinellids include those of Blackman (1967) and Őzder and Sağlam (2003) for *Adalia bipunctata* and *Coccinella septempunctata*, Michels and Behle (1991) for *Hippodamia sinuata* (in which the prey species effect on development rate disappeared at temperatures exceeding 20 °C) and Wiebe and Obrycki (2002) for *Coleomegilla maculata* (and the lacewing *Chrysoperla carnea*). Sadeghi and Gilbert (1999), Mendes et al. (2002) and Petersen and Hunter (2002) studied larval performance in the hover-fly *Episyrphus balteatus*, in the anthocorid bug *Orius insidiosus* and in lacewings respectively, in relation to prey species.

Albuquerque et al. (1997) investigated and compared growth and development (and also reproduction) in two lacewings, one a specialist, the other a generalist, examining what alterations in these variables occurred when the predator species were given each other's prey species (see their paper for details).

Two main factors influence how prey species can affect the growth and development of immature stages of predators. The first is based on the size of the prey species relative to the nutritional requirements of the predator. Smaller prey clearly contain fewer resources than larger prey. Furthermore, prey availability is important: regardless of prey size, optimal growth may only occur if there are sufficient prey encountered during immature growth. A second factor is prey quality, and this in turn may vary among different prey species. For example, Strohmeyer et al. (1998) found that the growth of two generalist predators, a stink bug (*Podisus maculiventris*) and jumping spider (*Phidippus audax*) varied among generalist, novel and specialist herbivore prey species reared on ribwort plantain (*Plantago lanceolata*) as well as on powder diets containing chemical extracts from new or mature leaves. The authors suggest that plant allelochemicals (iridoid glycosides) that were sequestered by the specialist herbivore but not the novel or generalist herbivores, may have impeded growth of the two predators, but that more factors were invariably responsible. Importantly, the effect of prey species on the growth of predators may be influenced by physiological interactions with plants across three trophic levels (Ode, 2006).

2.9.2.6 Interference and Exploitation Competition and Other Interference Effects

Ecologists distinguish between competition through interference and competition through exploitation. In interference competition individuals respond to one another directly rather than to the level to which they have depleted the resource. In exploitation competition individuals respond, not directly to each other's presence, but to the level of resource depletion that each produces. With exploitation competition the intensity of competition is closely linked to the level of the resource that the competitors require, but with interference it is often only loosely linked (Begon et al., 1996; Amarasekare, 2002).

Larval predators show interference in the form of behavioural interactions. For example, larval dragonflies may interfere with one another's feeding through distraction (e.g., 'staring encounters' between dragonflies) and/or overt aggression (Baker, 1981; McPeek & Crowley, 1987; Crowley & Martin, 1989; Fig. 2.46). Such interactions are likely to result in reduced feeding or increased metabolic costs and therefore ultimately will cause reduced growth, development and survival. Despite a superabundance of prey, interference competition between the native ladybird, *Coccinella undecimpunctata*, and the invasive ladybird, *Harmonia axyridis*, in the Azores led to greatly reduced prey consumption in the native species, which had knock-on effects on reproduction and maintenance of body mass (Soares & Serpa, 2007). Interestingly, interference competition was absent in the native ladybird species.

Van Buskirk (1987) conducted an experiment to test whether density-dependent, interference-mediated reductions in growth, development and survival occurred in larvae of the dragonfly *Pachydiplax longipennis*. First-instar larvae were raised in pools at initial densities of 38, 152 and 608 larvae/m^2, under two levels of prey availability (extra prey added daily to those already in pool; extra prey not added, i.e., food depletion likely to occur, pools in both cases containing the same initial density of prey), in a 3 × 2 factorial design. Van Buskirk (1987) found that with increasing predator density there was a decrease in growth and development rates, but he did not

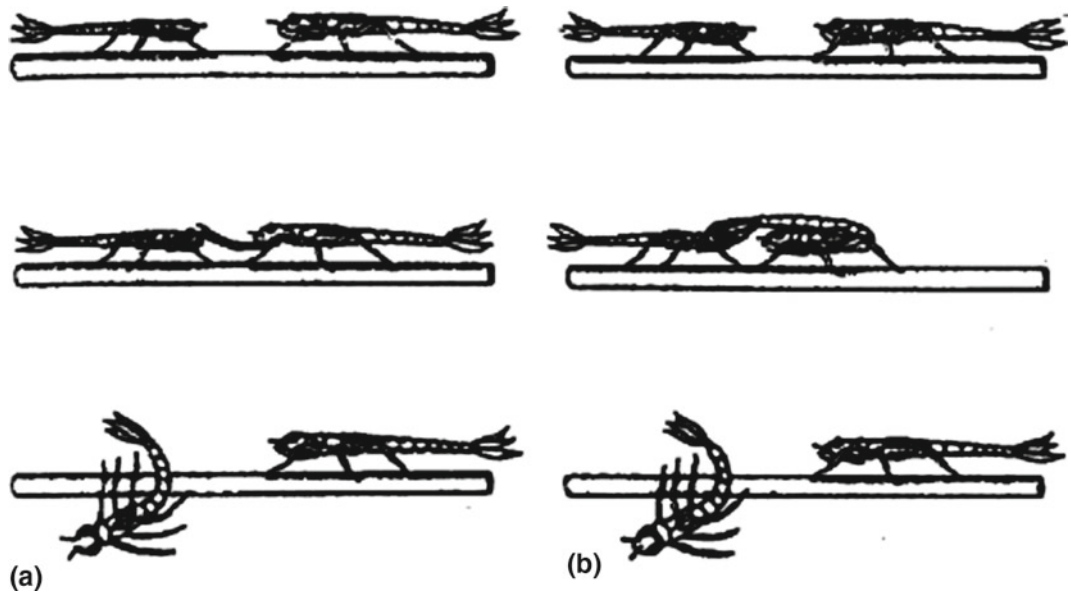


Fig. 2.46 Aggressive interactions between damselfly larvae: **a** labial striking; **b** slashing with the gills. Larger larvae usually displace smaller ones, which may retreat by swimming off the perch. *Source* Williams and Feltmate (1992). Reproduced by permission of CAB Publishing

detect any statistically significant interactions between prey addition and predator density, suggesting that some form of interference, rather than prey exploitation, was important. Within the prey-added treatment, the *per capita* amount of prey available was greatest at low predator densities (since identical amounts of prey were available at all predator densities). If larvae were competing by exploitation alone, prey availability would have had a greater positive effect at low predator densities than at high predator densities, but this did not show up in the statistical analyses. Instead, prey availability increased survival by a similar amount at all predator densities. The positive effect of prey availability on survival suggests that food stress in the prey-absent larvae led to their becoming cannibalistic, the assumption being that larval dragonflies can survive long periods of time without prey, and thus mortality could not be attributed to starvation (Lawton et al., 1980; Sect. 2.10). Direct evidence of cannibalism was not, however, obtained.

Baker (1989), using the 'condition' (an index of the relative mass per unit head width of larvae) of larval dragonflies, related larval growth to larval density in a series of field sites. He found that for most of the year there was little evidence of food limitation. His results are in contrast to those obtained in the study by van Buskirk (1987) and in studies by Pierce et al. (1985), Johnson et al. (1984) and Banks and Thompson (1987b), in which the data indicate aggressive interactions to be important in limiting food intake of larval Odonata in the field. Among the reasons for this discrepancy given by Baker (1989) are that in his study larval densities were not high enough for either exploitation competition or interference to occur. Baker (1989) also points to differences in methodology and interpretation between his study and those of other workers (see discussion in his paper).

Anholt (1990) points to 'asymmetries in the burden of refutation' in several studies of competition in larval Odonata and other animals. Authors, when they have been unable to find evidence of prey depletion, have concluded by default that interference is the primary cause of density-dependent growth, development and survival. That is, they have made the assumption that if it is not competition through exploitation, then it must be competition through interference. Anholt's (1990) study represents a significant departure from previous work on Odonata, in that he attempted to disentangle the effects of interference and exploitation by manipulating the rates of the two processes. Anholt manipulated the frequency of interactions between larval *Enallagma boreale* by altering perch availability at a fixed density of predators. Anholt (1990) argued that increasing the abundance of perches (i.e., increasing habitat complexity) will reduce the frequency of larva–larva encounters and thereby reduce the intensity of interference competition without affecting the supply of planktonic prey, i.e., without depletion occurring. Anholt's (1990) experimental design was a fixed-effects analysis of variance: (1) with three factors (food availability, larval density, perch availability) completely crossed; (2) with two factors (larval density and food availability) crossed; and (3) with two factors (perch availability and starting instar) crossed. In Anholt's (1990) experiments, damselflies became more evenly distributed among available perches as the predator density per perch increased, demonstrating that there were behavioural responses to the manipulation of habitat complexity (a prediction made by Crowley et al., 1987). Food supply and predator density strongly affected survival, but the proportion of the variance in survival attributable to the habitat complexity manipulation, i.e., interference, was very small. Furthermore, whilst there were significant density-dependent alterations in growth or development, they were not attributable to food-related interference competition. Thus, despite the overt nature of the interactions between individuals, their costs appear to be minimal. Anholt (1990) suggested that the density-dependent reduction in larval growth and development observed in his experiments could have been due to both 'resource depletion', i.e., exploitation, and resource depression. Resource depression is a term used to describe local reductions in prey availability that result from the

prey minimising the risk of predation by becoming less active and/or altering their use of habitat space.

Gribbin and Thompson (1990) conducted laboratory experiments in which individuals of two instars (ones which commonly occur together in the field) of *Ischnura elegans* were maintained in small containers (transparent plastic cups) with a superabundance of prey (to avoid prey limitation) either: (1) in isolation, (2) with three larvae of the same instar, or (3) three larvae of different instars. Either one perch or a set of four perches was provided to larvae in each treatment, and the experiment was treated as a two-way analysis of variance with perch availability as one factor and larval combination as the second factor potentially influencing development and growth. Small larvae showed increased development times and decreased growth (measured as percentage increase in head width) when kept with large larvae, but similar effects were not evident when the small larvae were kept with other small larvae. Development time and size increases of large larvae were not significantly affected by the presence of small larvae, i.e., competition was asymmetric. Regardless of the instar combination used, reductions in growth and development (which were taken to be due to interference, since prey—approximately 200 *Daphnia magna*—was superabundant in all treatments) were lessened when there were more perches available, although only in a few cases was the lessening significant. Gribbin and Thompson (1990) found that in containers with only one perch, large larvae often occupied the perch, whilst the single, small larva positioned itself on the side of the cup where feeding efficiency was likely to have been reduced.

Hopper et al. (1996) investigated the consequences of cannibalism for growth and survival (Sect. 2.10.2) of survivors in the dragonfly *Epitheca cynosura*. The eventual size of survivors from a high larval density, asynchronous treatment (asynchronous in hatching terms—asynchrony increases the likelihood of cannibalism, i.e., by older larvae) was greater than that of survivors from a low larval density, asynchronous treatment, while there was no difference in size between survivors from high and low larval density synchronous treatments.

For a study of interference and exploitation competition in a species of carabid beetle, see Griffith and Poulson (1993). Interference competition has been shown by Griffiths (1992) to occur between larvae of the ant-lion *Macroleon quinquemaculatus*. Note that facilitation, not interference, may occur between larval conspecifics in some predator species, e.g., nymphs of the pentatomid *Perillus bioculatus* (Cloutier, 1997).

Exploitation competition for lime aphid prey between the ladybird *Harmonia axyridis* and the predatory flowerbug *Anthocoris nemoralis* reduced the presence of lime aphid DNA detected in the bodies of *A. nemoralis* (Howe et al., 2016). Exploitation competition is apparently greater than intraspecific competition among three species of native, alien and invasive ladybirds in Chile (Zaviezo et al., 2019). Exploitation competition between two species of hover-fly was studied by Hågvar (1972, 1973).

The deleterious effects of competition on larval growth (and fecundity) can be expressed by plotting *k*-values (defined in Sect. 7.3.4) against $\log_{10}$ predator density. When describing such effects, the terms 'scramble' and 'contest' competition are less appropriate than the terms 'exact compensation', 'over compensation' and 'under compensation' (Begon et al., 1996; Sect. 7.3.4).

Larvae may also show a reduction in feeding rates in the presence of higher-level predators (Murdoch & Sih, 1978; Sih, 1982; Heads, 1986). Such interference may reduce the rate of consumption of prey, even when the insects do not need to move in order to feed (Heads, 1986), with the potential result that growth, development and even survival may be adversely affected (Sih, 1982; Heads, 1986; see, however, Brodin & Johansson, 2002). McPeek et al. (2001) showed that although the larvae of *Ischnura* and *Enallagma* ingest less food in the presence of a fish predator, interspecific differences in growth rate were primarily due to differences in the conversion efficiency of the species, i.e., the two genera differ in their physiological stress response to the

presence of predators. Stoks (2001) concluded from his study of *Lestes sponsa* that predator-induced stress effects upon growth and development were due to lowered assimilation efficiency and/or a higher metabolic rate.

The early-instar larvae of the waterboatman *Notonecta hoffmani* can suffer significant mortality due to predation from adult conspecifics (Murdoch & Sih, 1978; Sih, 1982), and the adult avoidance behaviour of larvae constitutes a form of interference. Sih (1982), in laboratory and field experiments, compared the behaviour of larvae when the adults were experimentally removed with their behaviour in controls where adults were present. Early-instar larvae avoided adults by altering their use of habitat space (spending less of the total time available in the central region of the pond or tub, where prey and adults occur at the highest densities), and some of the early instars also became less active. As a result of this behaviour, larvae of the first two instars experienced severely reduced feeding rates.

2.9.2.7 Host Size

Idiobionts

The concept of an individual host as a fixed 'parcel' of resource for a developing idiobiont parasitoid was introduced in Chap. 1. For many idiobiont species host size determines the size (and/or mass) of the resultant parasitoid adult(s), as shown by data both on solitary and on gregarious species (Salt, 1940, 1941; Arthur & Wylie, 1959; Heaversedge, 1967; Charnov et al., 1981; Greenblatt et al., 1982; Waage & Ng, 1984; van Bergeijk et al., 1989; Corrigan & Lashomb, 1990; Otto & Mackauer, 1998; Harvey et al., 2006; Harvey, 2008; Wei et al., 2014; reviewed by Godfray, 1994 and Harvey, 2005). Idiobiont parasitoids, by virtue of attacking hosts that do not feed or grow, have evolved to exploit the trophic level below them (herbivores for primary parasitoids and primary parasitoids for hyperparasitoids) with remarkable efficiency. For example, Harvey et al. (2006) found newly emerged adults of the primary koinobiont parasitoid *Cotesia glomerata* were only marginally larger than newly emerged adult hyperparasitoids of the idiobiont species *Lysibia nana* that had developed in *C. glomerata* (pre)pupae of equivalent size. This efficiency allows food chains to be extended to five or even more trophic levels (Harvey et al., 2009a, b). Development rate, however, is not necessarily positively correlated with the size of host oviposited in. For example, in *Trichogramma evanescens*, development rate is highest in medium-sized eggs and lowest in small and large eggs (Salt, 1940), in *Elachertus cacoeciae* it is highest on fifth-instar hosts and lower in fourth and sixth instars (Fidgen et al., 2000), in *Goniozus nephantidis* it is lowest in seventh and eighth instars of the natural host, *Opisina arenosella*, weighing >70 mg and highest in the factitious host, *Corcyra cephalonica* (Shameer et al., 2002) while in *Habrobracon hebetor* development time is unaffected by host larval size (Taylor, 1988). The reasons for the lack of a clear relationship are complex, and the reader is referred to Mackauer and Sequeira (1993).

To investigate the influence of host size on growth and development in an idiobiont parasitoid species, present females (inseminated and, if necessary, uninseminated, to obtain data on both sexes) with hosts of different sizes and record the weight of the resultant adult progeny and the time taken from oviposition to adult eclosion (since adult eclosion is often influenced by light: dark cycles [Mackauer & Henkelman, 1975], observations should be carried out at the same time each day or under continous light conditions; video-recording equipment can be used both to improve accuracy and to save time [Sequeira & Mackauer, 1992a]). If the parasitoid is a gregarious species, clutch size will have to be kept constant (Sect. 1.10. describes clutch size manipulation techniques). One needs to bear in mind the possibly complicating effects of sex differences in food acquisition (and therefore growth and development) in broods of gregarious species. This problem can be partly circumvented by using uninseminated parent females, which

will produce all-male egg clutches, but obtaining all-female clutches could prove very difficult (Sect. 1.10).

For idiobiont parasitoids the age of the host may be a confounding factor. For example, some parasitoids that develop in host pupae may be able to utilise both very recently formed pupae and pupae within which the adult host is about to be formed. These different types of host pupa are likely to have the same external dimensions and similar mass but are likely to represent very different amounts of resource. As host pupae age, their bodies undergo radical morphological and physiological changes in a comparatively short period of time. This includes differentiation into various body structures such as wings, the head and thorax, appendages, and sclerotisation of the cuticle, that may also affect the amount of resource available to the larvae of idiobionts. For this reason, older pupae often are of lower quality for the development of idiobionts than pre-pupae or young pupae (Harvey, 2005). Similarly, older egg are often less suitable and less preferred hosts for egg parasitoids than younger eggs. This may be because older eggs contain more fully developed embryos that are more difficult to consume and assilimate (Pizzol et al., 2012).

To determine whether host stage i.e., instar and not host size per se mainly accounts for any variation in growth or rate of development, parasitoids, e.g., idiobionts attacking larval Lepidoptera, can be presented with a range of host sizes within each host stage that overlaps with host sizes within the previous or subsequent stage.

Working over four trophic levels, Otto and Mackauer (1998) compared development of the idiobiont hyperparasitoid *Dendrocerus carpenteri* in its primary host *Aphidius ervi* which itself was reared in two aphid host species (*Acyrthosiphon pisum* and *Sitobium avenae*) of differing quality and growth potential. Within each aphid species, the authors found that terminal host size affected the size of *A. ervi*, which had a concomitant effect on the size of *D. carpenteri*. However, the development time of the hyperparasitoid was determined by the age of the *A. ervi* individual when it was attacked, and was longer in older hosts, which presumably was attributable to their reduced digestibility.

Koinobionts

During the initial phases of parasitism, hosts of koinobionts remain active and may continue feeding, growing and defending themselves (Mackauer and Sequiera, 1993). Thus, for a koinobiont the host represents a dynamic resource. One might therefore not expect the same relationship between progeny size and host size at oviposition as exists for idiobionts (Godfray, 1994; Mackauer, 1986; Harvey, 2000, 2005).

Sequeira and Mackauer (1992a, b) and Harvey et al. (1994, 1999, 2004) have shown, for different koinobionts, that adult parasitoid size (mass) is not a linear function of host size (mass) at oviposition across the full range of available host sizes (Fig. 2.47a). In the solitary parasitoids *Aphidius ervi* and *Venturia canescens*, there is a linear increase in wasp size with increasing instar up to the penultimate instar, whereas in the final instar wasp size does not increase. By contrast, in *Cotesia rubecula*, which is also solitary, adult wasp size more generally decreases with instar parasitised. These variations in koinobiont development are linked to differences in host usage strategy (discussed by Harvey et al., 2000b; Harvey, 2005; Harvey & Malcicka, 2016). Whereas the larvae of most koinobionts obligatorily consume most (or all) host tissues before pupation, several endoparasitoid clades contain taxa whose larvae primarily consume host haemolymph before emerging through the side of a still-living host, to pupate externally. Since in the latter group only a fraction of the available host resources is consumed, the relationship between host size and parasitoid size may be more complex than for tissue-feeders. For example, parasitoid development may be more constrained by the availability of certain nutrients in the host haemolymph, rather than by host size per se.

The relationship between development rate and the size of the host when parasitised varies, being either linear throughout the whole range of available host sizes and highest in larger hosts (Fox et al., 1967; Smilowitz & Iwantsch, 1973; Harvey et al., 2000b) or non-linear (Jones &

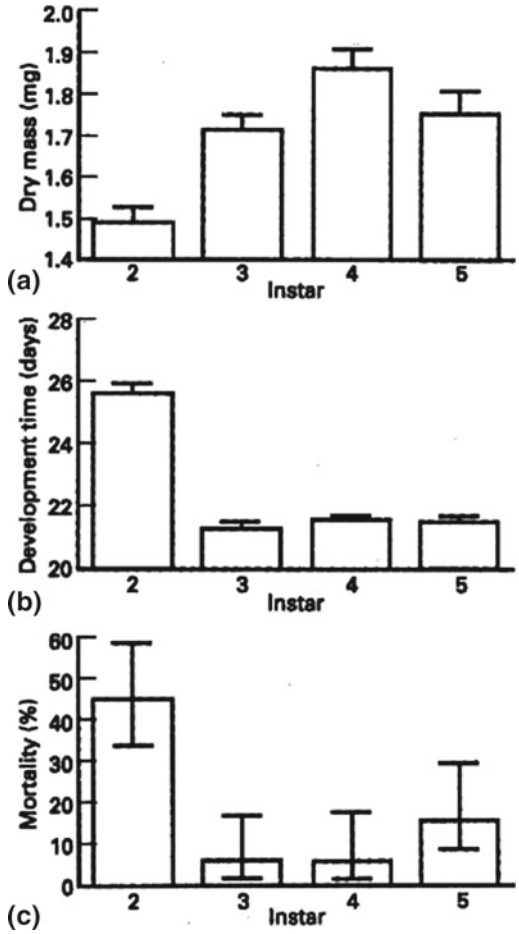


Fig. 2.47 Growth, development and mortality of *Venturia canescens* (Ichneumonidae) reared in four instars of the moth *Plodia interpunctella*. **a** Adult dry mass; **b** development time from oviposition to adult eclosion; **c** mortality. Bars represent standard errors of the mean. *Source* Harvey et al. (1994). Reproduced by permission of The Ecological Society of America

Lewis, 1971; Avilla & Copland, 1987; de Jong & van Alphen, 1989; Harvey et al., 1994, 2000b; Fig. 2.47b).

The relationship between host size and immature parasitoid development rate has been shown to differ even among closely related koinobiont species. For instance, whereas *Venturia canescens* delays development in early instars, a closely related species *Campoletis sonorensis* (both parasitoids are in the ichneumonid subfamily Campopleginae) develops at fairly similar rates to eclosion in different host instars (Harvey & Strand, 2002). These differences appear to reflect constraints imposed by the final size of the host relative to adult parasitoid size, as well as host growth rate. Whereas *V. canescens* habitually attacks comparatively small caterpillars of micro-lepidopteran hosts which grow slowly, *C. sonorensis* parasitises larger caterpillars of macro-lepidopteran hosts that grow quite rapidly. Moreover, differences in host usage strategies among koinobionts also influence development. Whereas the larvae of most koinobionts consume virtually the entire host piecemeal before pupating (= tissue-feeders), a small number of species in several braconid subfamilies (e.g., Microgastrinae, Cheloninae) feed primarily on host haemolymph, leaving most host tissues intact when they are fully grown (= haemolymph-feeders; Harvey, 2005; Harvey & Malcicka, 2016). The mature larvae perforate the host cuticle with specialised mandibles (Nakamatsu et al., 2006) and pupate externally, either spinning cocoons under the host body or attached to the host cuticle (Harvey et al., 2008a, b; Quicke, 2014). Species of tissue- and haemolymph-feeders can be found in the braconid subfamily Microgastinae (Harvey et al., 2000b; Harvey & Gols, 2018). The parasitised host is often 'usurped' by haemolymph-feeders as a surrogate bodyguard to protect the parasitoid cocoons against natural enemies such as predators and hyperparasitoids (Grosman et al., 2008; Harvey et al., 2008a, b, 2011; Mohan & Sinu, 2017) or as an alternate, more nutritionally valuable source of prey for predators (Harvey et al., 2013a, b).

Valuable insights into the effects of host stage on growth and development can be obtained by plotting the growth trajectories of both the host and the parasitoid (Sequeira & Mackauer, 1992b; Harvey et al., 1994, 1999; Harvey & Strand, 2002; Fig. 2.48). Growth trajectories are studied by taking each host stage, dissecting parasitised hosts at various points in time after oviposition, separating the parasitoid larva from the host and measuring the dry weight of each. A trajectory is also plotted for unparasitised hosts. Using growth trajectories, Sequeira and Mackauer (1992b) showed that *A. ervi* responds to host-related

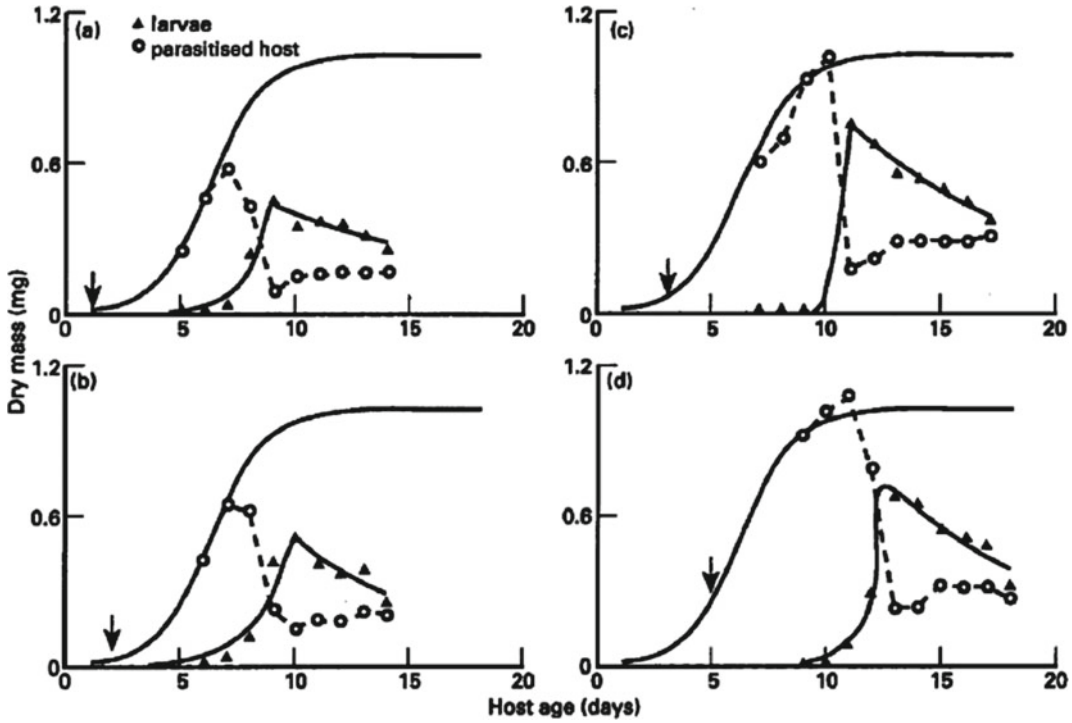


Fig. 2.48 Growth trajectories of *Aphidius ervi* (▲) and of parasitised pea aphids (○) at different ages: **a** host nymphal instar one (24 h); **b** host nymphal instar two (48 h); **c** host nymphal instar three (72 h); **d** host nymphal instar four (120 h). The solid curve shows the corresponding trajectory of unparasitised aphids, samples of which were taken at various ages from birth to maturity. Arrows indicate the age of the host at oviposition. The 'turnover' point of the parasitoid growth trajectory corresponds to parasitoid age of 8 days. The trajectory of parasitoid larval growth provides a direct measure of host 'quality', reflecting the nutritional relationship between the two insects during the course of parasitism, and its shape will be characteristic of the parasitoid species. All curves will, however, be 'J'-shaped, there being two functionally distinct phases in the development of holometabolous insects: first there is an exponential growth phase as the parasitoid larva feeds and converts host tissues into its own body mass, then there is a negative exponential decay phase between pupation and adult eclosion, when feeding has stopped and there is differential mass reduction due to respiration, water loss and voiding of the meconium (Harvey et al., 1994). *Source* Sequeira and Mackauer (1992b). Reproduced by permission of The Ecological Society of America

constraints upon larval growth, and arrests host growth at a largely fixed time approximately 8 days after oviposition, at which point aphids parasitised as early instars have not reached their maximum size. In *A. ervi*, development time and adult mass covary positively (i.e., there is a trade-off between development rate and growth) with an increase in host size from first to third instar, but they vary independently in parasitoids developing in fourth-instar hosts. In the latter, adult mass does not increase but development rate does. Overall parasitoid development time is therefore approximately constant, whereas the largest wasps emerge from third- and fourth-instar aphids. The growth trajectories shown in Fig. 2.48 indicate that in early-instar hosts parasitoid growth and development rate are limited by the small size and growth potential of the host (compare, in Fig. 2.48, the average mass attained by parasitised aphids with that attained by unparasitised aphids of equivalent age). By contrast, in fourth-instar hosts excess resources are constantly available,

thus allowing for an increase in development rate without an increase in adult mass.

As pointed out by Harvey et al. (1994), *A. ervi* may represent one end of a continuum of strategies among parasitoids, the other extreme being to delay parasitoid growth until the host reaches its maximum size (in which case we would expect parasitoid size to be unaffected by instar at oviposition but development rate to be highly variable). The latter pattern is exhibited by *Apanteles carpatus*, which attacks a wide range of sizes (representing all larval instars) of its host, the clothes moth *Tineola bisselliella*. Irrespective of host size at oviposition, the size of emerging wasps is close to uniform, whereas development time increases exponentially with a decrease in host size, some wasps taking three months to complete their development in very small hosts (Harvey et al., 2000b). The strategy of *V. canescens* appears to lie somewhere along the continuum between the aforementioned two extremes (Harvey et al., 1994; Harvey & Vet, 1997). See Harvey and Strand (2002) for a review of parasitoid developmental strategies.

As these studies have shown, by comparing the development of koinobionts in very small or otherwise nutritionally suboptimal hosts, it should be possible to elucidate the nature of trade-offs between life-history variables. The experimental protocol for studying the effects of host stage at oviposition upon growth and development is slightly more complex for koinobionts than for idiobionts inasmuch as the hosts need to be reared. Care must be taken to control for the effects of variations in host diet; Harvey et al. (1994), for example, reared hosts with an excess of food. However, as pointed out by Mackauer and Sequeira (1993), there is a need to examine the dynamics of parasitoid development under different constraints. These might include superparasitism, particularly in the case of gregarious species, where crowding intensifies competition with conspecifics for access to limited host resources (Wajnberg, et al., 1990; Harvey, 2000). There is also a need for more studies on the nutritional integration between host and parasitoid when hosts are reared on various food plants containing different concentrations of constitutively expressed or induced defensive chemical compounds (see below).

2.9.2.8 Host Species

Given that hosts of different species are likely to constitute different resources, in both qualitative and quantitative senses, we would expect parasitoid growth and development to vary in relation to the host species parasitised. This is indeed the case, as studies with both idiobionts and koinobionts have shown (although few workers have measured growth together with development) (Taylor, 1988; Ruberson et al., 1989; Corrigan & Lashomb, 1990; Harvey & Thompson, 1995; Harvey & Vet, 1997; Harvey & Gols, 1998; Harvey et al., 1999, 2010, 2015; McNeill et al., 1999; Nicol & Mackauer, 1999; Eben et al., 2000; Seal et al., 2002; Shameer et al., 2002; Bazzocchi et al., 2003; Pérez-Lachaud et al., 2004; Harvey, 2005; Milonas, 2005; Häckermann et al., 2007; Ghimire & Phillips, 2014; Lupi et al., 2017; Abdi et al., 2021).

Salt (1940), for example, showed how the size of adult progeny of *Trichogramma evanescens* varied with the species of moth within which larval development occurred (Fig. 2.49). Moratorio (1987), working with *Anagrus mutans* and *A. silwoodensis*, showed that female progeny were larger when development occurred in the (large) eggs of *Cicadella viridis*, but were smaller when development occurred in the (small) eggs of *Dicrantropis hamata*. However, whereas *A. silwoodensis* develops fastest in *C. viridis*, *A. mutans* develops fastest in *D. hamata*, i.e., development rate and growth countervary in relation to host species in *A. mutans*. Development rate and growth also countervary in relation to host species in *Telenomus lobatus* (Scelionidae): wasps develop more rapidly in eggs of *Chrysoperla* species than in eggs of *Chrysopa* species, but the adults attain a larger size in eggs of the latter genus, the eggs being larger than those of *Chrysoperla* (Ruberson et al., 1989).

Similar findings have been reported in some koinobionts. In *V. canescens*, adult wasp size is positively correlated with the growth potential of the particular host species, although development

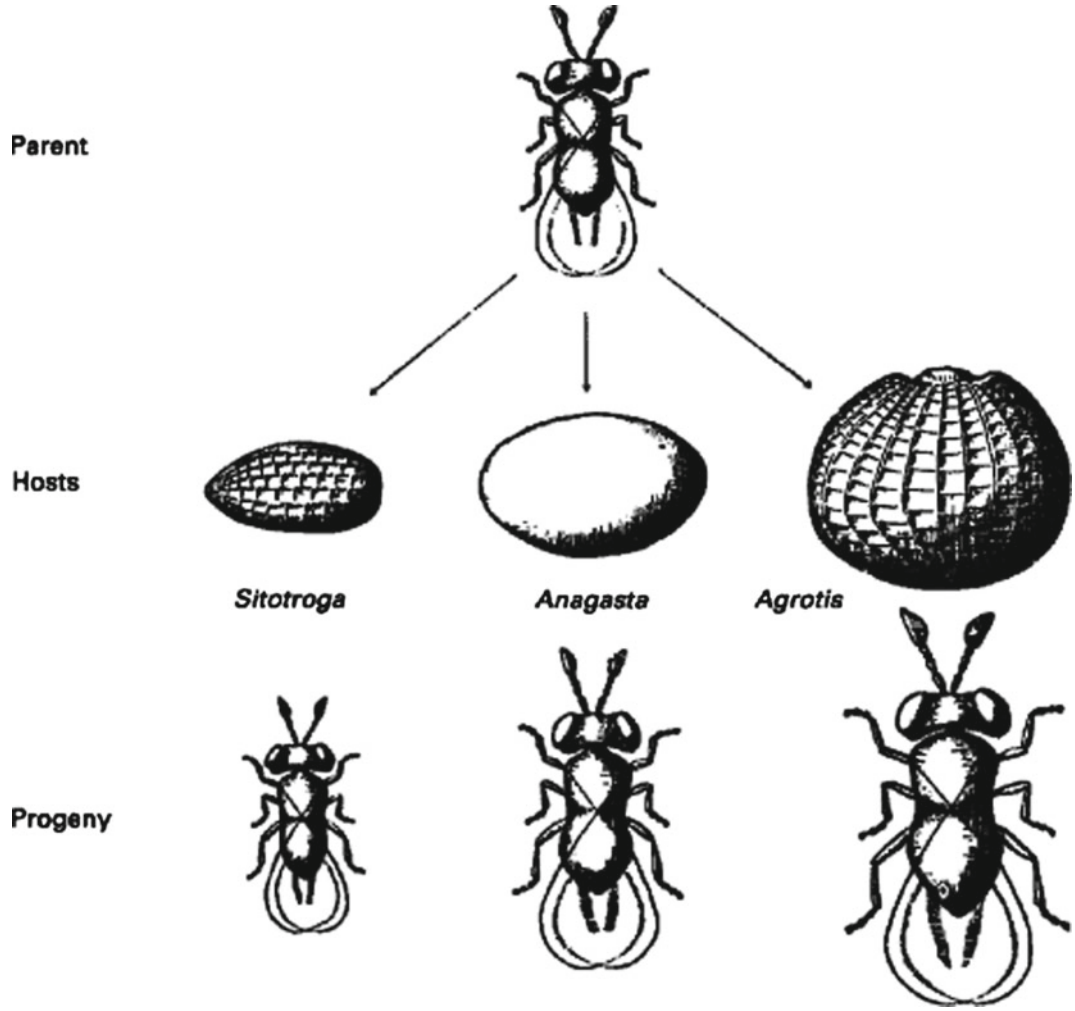


Fig. 2.49 The relative sizes of female *Trichogramma evanescens* (Trichogrammatidae) and female progeny reared from different host species. The reader should note that the confounding effects of progeny clutch size were not controlled for in Salt's (1940) experiments (development was solitary in *Sitotroga* and *Anagasta* (now *Ephestia*) but was either solitary or gregarious in *Agrotis*). The female that emerged from the egg of *Agrotis* developed solitarily; nevertheless, females that developed gregariously in that host species were on average markedly larger than those that developed in either of the other two host species. *Source* Salt (1940). Reproduced by permission of Cambridge University Press

time is extended in larger hosts (Harvey & Thompson, 1995; Harvey & Vet, 1997). By contrast, in *C. rubecula*, emerging wasps are larger and develop more rapidly in a smaller, habitual host (*Pieris rapae*), than in corresponding stages of a larger, factitious host (*P. brassicae*, Harvey et al., 1999). Harvey et al. (1999) found that *C. rubecula* arrested the development of *P. brassicae* larvae at an earlier stage (and smaller size) than that of larvae of *P. rapae*. This effect could be related to the fact that *P. rapae* is generally a much more suitable host for *C. rubecula*, which is rarely recovered in the field from other host species, including *P. brassicae*. Further investigations should focus on the potential influence of host species on parasitoid development among host species of equivalent size (mass). If differences in

performance are recorded, then this would suggest that the quality, rather than the quantity, of host resource affects growth and development.

2.9.2.9 Multitrophic Interactions and the Performance of Natural Enemies

It is well established that plants play an important role by mediating a suite of physiological interactions amongst the herbivores feeding on them and the natural enemies of the herbivores. Plants contain a bewildering array of toxic secondary compounds (Karban & Baldwin, 1997; Schoonhoven et al., 2005), some of which negatively affect the herbivore's growth, development and survival (van Dam et al., 2000; War et al., 2012; Nishida, 2014; Kessler & Kalske, 2018; Gajger & Dar, 2021). These toxins are also frequently sequestered in the haemolymph or body tissues of resistant herbivores, thus providing them with the potential for some degree of protection against their natural enemy complex (Tullberg & Hunter, 1996; Wink et al., 2000; Omacini et al., 2001; Erb & Robert, 2016; Petscheka and Agrawal, 2016).

Many studies have reported that allelochemicals in the diet of the prey or host can negatively affect the growth, development, survival or morphology of their predators and parasitoids (Barbosa et al., 1986; Duffey et al., 1986; Gunasena et al., 1990; Paradise & Stamp, 1993; Karban & English-Loeb, 1997; Havill & Raffa, 2000; Harvey et al., 2005, 2007; Lampert et al., 2010; Zimmerman et al., 2021; reviewed by Turlings & Benrey, 1998; Ode, 2006, 2019). In some cases, one of the aforementioned life-history variables is negatively affected whereas another is not (Karban & English-Loeb, 1997), and allelochemicals may reduce development rate and growth rate only when prey are scarce (Weisser & Stamp, 1998). The effects of interspecific variation in plant quality may even work their way up to organisms in the fourth trophic level, such as primary parasitoids of insect predators or obligate hyperparasitoids (Orr & Boethel, 1986; Harvey et al., 2003, 2007).

Harvey et al. (2003) and Fritz et al. (1997) demonstrated differences in the performance of parasitoids depending on host plant quality. Schädler et al. (2010) observed that the effects of genotypes across trophic levels are more complex than the argument that high-quality plants produce high-quality herbivores with positive effects on higher trophic levels. The plant genotypes may have significant effects on the performance of herbivores, but the influence of plant genotype on predators and parasitoids is weaker than on herbivores (Schädler et al., 2010). In similar experiments, Shameer (2017) reported differences in larval, pupal and egg-to-adult period of the lepidopteran herbivore *Opisina arenosella* feeding on the leaves of different varieties/hybrids of coconut, but for the parasitoid *Goniozus nephantidis* reared on these hosts, developmental timing differences were observed only in the pre-oviposition and pupal periods. However, the egg-to-adult survival of the parasitoid was affected by the variety of coconut on which the host had fed. Similarly, the plant genotypes on which *Plutella xylostella* were reared affected the developmental times of both females and males of the parasitoid *Diadegma insulare* (Cresson) (Ichneumonidae) (Sarfraz et al., 2008). This may be due to the presence or absence of specific nutrients in the host's diet, the presence of detrimental allelochemicals, or an interaction between nutrients and allelochemicals (Turlings & Benrey, 1998).

Experiments can be conducted in which growth and development of predators and parasitoids are measured when the carnivores are reared on separate cohorts of hosts or prey that have been fed on resistant and non-resistant strains of a cultivated plant, or on related species of wild plants. Of particular interest is the degree of adaptation shown by adapted specialist herbivores and their parasitoids, which in some cases perform better on more toxic plant species or genotypes (Harvey et al., 2003). The effects of plant secondary compounds can also be investigated by incorporating the chemicals into the artificial diet of the herbivore (e.g., Campbell & Duffey, 1979; Williams et al., 1988; Reitz & Trumble, 1997; Weisser & Stamp, 1998). Small amounts of a compound added to such a diet may even improve parasitoid larval performance (Williams et al., 1988; Harvey et al., 2007). It

should also be noted that fungal endophytes produce toxins that may affect larval parasitoid growth and/or development (e.g., Barker & Addison, 1996; Bultman et al., 1997).

2.9.2.10 Superparasitism, Multiparasitism, and Intrinsic Competition

Introduction

Superparasitism is defined as the laying of an egg (by a solitary parasitoid) or a number of eggs (by a gregarious parasitoid) in (or onto) an already parasitised host (Sect. 1.9.4). In the case of a solitary parasitoid species, only one larva per superparasitised host survives. In a gregarious species the number of survivors per host will depend on the total number of eggs laid and the size of the superparasitised host (Beckage & Riddiford, 1978; le Masurier, 1991). In multiparasitised hosts, two species of parasitoids compete for host resources, and in solitary species, only one species survives. In gregarious parasitoids, both species may survive multiparasitism, but otherwise the effects on survival and development are generally similar to those observed with superparasitism (Harvey et al., 2013a, b; Cusumano et al., 2016). This section is concerned with ways of studying the fitness consequences for surviving larvae, and asks how larval growth and development rate might be affected by superparasitism.

Solitary Parasitoids

Models of superparasitism as an adaptive strategy in solitary species (van der Hoeven & Hemerik, 1990; Visser et al., 1990) have been based on the assumption that superparasitism has no fitness consequences for the surviving larva, i.e., it does not increase larval development time or reduce adult size. This would seem to be a reasonable assumption, since in solitary parasitoids supernumerary larvae (larvae in excess of the number that can ultimately survive, i.e., can complete development) are usually eliminated before they can utilise an appreciable amount of host resource. For example, Visser et al. (1992) found no convincing evidence that *Leptopilina heterotoma* adults emerging from singly parasitised hosts were larger than adults emerging from superparasitised hosts (see also Ueno, 1997). However, as pointed out by Bai and Mackauer (1992) and Harvey et al. (1993), superparasitism may have fitness consequences for the larvae of some parasitoid species. Simmonds (1943) and Wylie (1983), for example, reported that in *Venturia canescens* (Ichneumonidae) and *Microctonus vittatae* (Braconidae) larvae take longer to develop in superparasitised hosts than in singly parasitised hosts, although neither author recorded the number of eggs contained per host. Similarly, Vinson and Sroka (1978), subjected hosts of *Cardiochiles nigriceps* (Braconidae) to varying numbers of ovipositions, recorded the time taken from oviposition to larval emergence from the host, and showed that as the degree of superparasitism increased, mean development time of the surviving larva increased (Table 2.1).

The fitness cost to koinobionts may be partly determined by the ability of the surviving larva to compensate for possibly reduced growth during

Table 2.1 Percentage of hosts yielding a larva, and the time taken from oviposition to larval emergence from the host, in the solitary parasitoid *Cardiochiles nigriceps* (Braconidae) parasitising *Heliothis virescens*. *Source* Vinson and Sroka (1978)

Number of ovipositions per host	% of hosts yielding a parasitoid	Mean time (days) to emergence
1	92	12.3 ± 1.6
2	58	12.2 ± 1.9
3	63	14.7 ± 2.7
4	29	15.6 ± 2.5
5	27	15.9 ± 3.0
>5	21	16.9 + 3.4

embryonic and early larval development (when it may compete with the rival larva for host resources) by increasing growth later in development (Bai & Mackauer, 1992), and the same might apply to development rate. Bai and Mackauer (1992) carried out a simple experiment in which they subjected aphids to either one oviposition (singly parasitised) or several ovipositions (superparasitised) by *Aphidius ervi*. They used unmated females, in order to control for the possible bias resulting from differential development (and survival) between male and female larvae. They then compared the total development time and adult weights in the different treatments. They found that *Aphidius ervi* gained 14% more dry mass in superparasitised hosts, i.e., growth was enhanced through superparasitism, and took no longer to develop, i.e., development rate was unaffected. The most likely explanation for this effect is that the superparasitised hosts ingested more food. As Bai and Mackauer (1992) point out, the fitness benefit, i.e., increased adult size gained by surviving larvae in superparasitised hosts, needs to be weighed against any costs in the form of reduced larval survival (Sect. 2.10.2).

As we noted above, adult size in *Leptopilina heterotoma* is not affected by superparasitism. In this parasitoid either compensation is complete or there is no initial reduction in growth as a result of superparasitism. Studying the trajectory of parasitoid larval growth (see above) would shed light on this.

Multiparasitism has also been shown to incur fitness costs on the surviving parasitoid. For example, in host (*Pseudoplusia includens*) caterpillars multiparasitised by two species of solitary *Microplitis* (Braconidae) parasitoids, development time was sometimes longer and adult body mass smaller in emerging wasps compared with controls (Harvey et al., 2009a, b). Similar results have been observed in egg parasitoids (Cusumano et al., 2015). In some multiparasitised hosts, one parasitoid species dominates, but offspring sex ratio becomes skewed towards male progeny (Walker et al., 2016; Luo et al., 2018). This, however, often depends on temporal differences in the sequence of the first and second ovipositions.

Experiments aimed at investigating the effects of superparasitism and multiparasitism on larval growth (as measured by adult size) and development would involve exposing a recently parasitised host to a standardised female and allowing the same or a different (conspecific or heterospecific) standardised female to deposit a specified number of eggs. The number of eggs laid in each case can be more easily monitored and controlled if the parasitoid is one of those species in which the female performs a characteristic movement during oviposition (Harvey et al., 1993; Sect. 1.11.6). The time taken from oviposition to adult eclosion and the size or weight of emerging adults will need to be measured and the different treatments compared both with one another and with controls. The experiment could be expanded to take into account the possible effects of host size or host instar, as was done by Harvey et al. (1993). They showed that superparasitism in *Venturia canescens* reduced development rate in parasitoids reared from both third-instar and fifth-instar larval hosts (the moth *Plodia interpunctella*), but that the reduction was greater in parasitoids reared from the later instar (Fig. 2.50). The size of wasps reared from third-instar hosts was unaffected by egg number (Fig. 2.51a), but adult wasps from both of the superparasitised fifth-instar treatments (two eggs, four eggs) were significantly smaller than those reared from singly parasitised hosts (Fig. 2.51b). Harvey et al. (1993) suggested that the reason superparasitism affected parasitoids from fifth-instar hosts more than those from third-instar hosts is that the fifth-instar larvae were post-feeding, wandering larvae, i.e., their growth potential is zero. Parasitism of such hosts would be more like idiobiosis than koinobiosis, and the surviving larva would be less able to compensate for any negative effects of superparasitism.

Gregarious Parasitoids

The fitness consequences of superparasitism have already been touched upon, from both theoretical and experimental standpoints, in Chap. 1

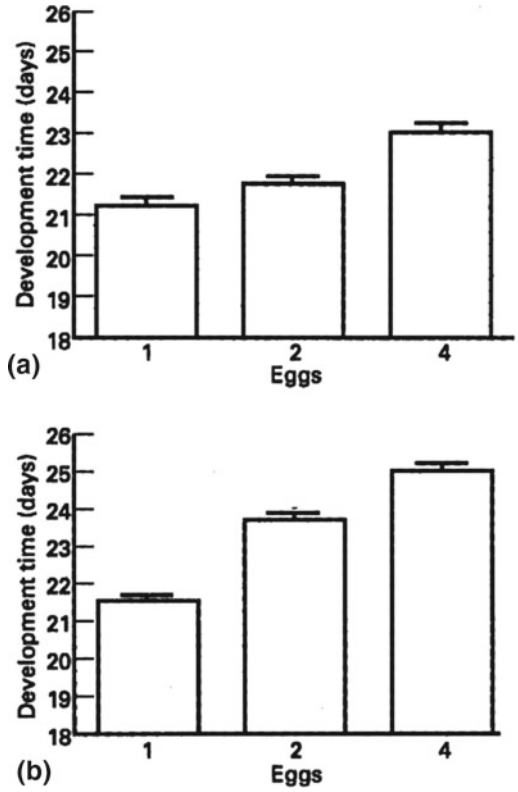


Fig. 2.50 Average (± SE) effects of superparasitism on development in the solitary ichneumonid parasitoid *Venturia canescens*. Development time (number of days taken from oviposition to adult eclosion) of wasps reared from: **a** third-instar **b** fifth-instar larvae of *Plodia interpunctella* containing one, two or four parasitoid eggs. *Source* Harvey et al. (1993). Reproduced by permission of Blackwell Publishing

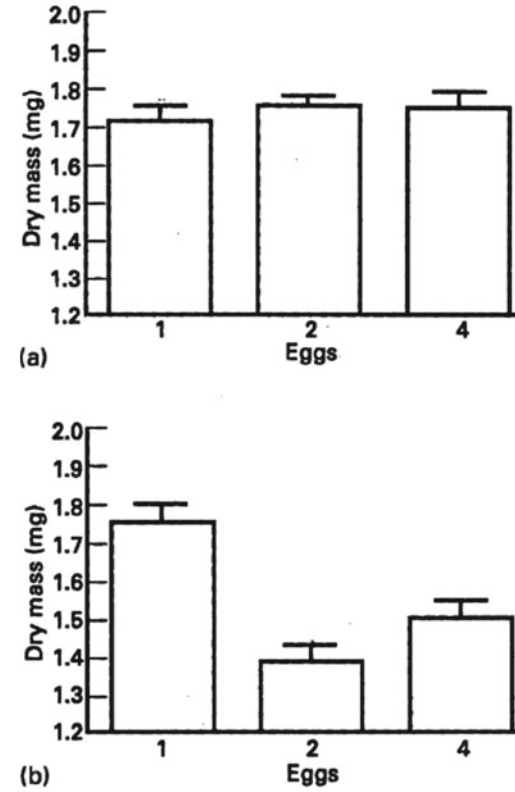


Fig. 2.51 Average (± SE) effects of superparasitism on growth (as measured by adult dry mass) in the solitary ichneumonid parasitoid *Venturia canescens*. The dry weight of wasps reared from: **a** third-instar **b** fifth-instar larvae of *Plodia interpunctella*, containing one, two or four eggs. *Source* Harvey et al. (1993). Reproduced by permission of Blackwell Publishing

(Sect. 1.9.4). Leaving aside Allee effects (defined in Sect. 1.10), superparasitism and multiparasitism will intensify competition among larvae for host resources, with the result that the *per capita* growth and development rate of the parasitoid immatures will be reduced. This is at least what one would expect, although Nealis et al. (1984) found that increased larval density per host slowed development of *Cotesia glomerata* only slightly (le Masurier, 1991, found no significant effect of clutch size on development time in this species) and tended to increase the rate of development in *Pteromalus puparum*. Le Masurier (1991) also found no significant decrease in body size with increasing clutch size in a population of *C. glomerata* parasitising *Pieris brassicae*, although he did find such an effect in another population parasitising *Pieris rapae*.

The fitness consequences for surviving gregarious parasitoids in the case of multiparasitism have thus far received much less attention than in the case of superparasitism. However, in haemolymph-feeding koinobiont endoparasitoids, much of the host is not consumed by the parasitoid larva(e), reducing the intensity of competition. In some species (e.g., Microgastrinae), this has enabled two different gregarious species to emerge from the same host, a phenomenon described as 'resource sharing' (Harvey et al., 2013a, b), but with some fitness-

related costs. For example, when caterpillars of *Mythimna separata* are multiparasitised by *Cotesia kariyai* and *C. ruficrus*, both parasitoids are able to successfully emerge (Magdaraog et al., 2012). However, brood sizes of both species are significantly less than in singly parasitised hosts. In intrinsic competition between the solitary endoparasitoid *Microplitis mediator* and the polyembryonic parasitoid *Copidosoma floridanum*, the latter species always wins (Strand et al., 1990). However, *C. floridanum* shifts investment from reproduction to defence by reallocating resources to the production of soldier larvae (and the production of less reproductive larvae) in multiparasitised hosts (Harvey et al., 2000a).

Experiments aimed at investigating the effects of superparasitism on larval growth (as measured by adult size) and development in gregarious parasitoids would involve: (1) in the case of intraspecific superparasitism, exposing a recently parasitised host to a standardised female and allowing the same or a different conspecific female to oviposit a further egg or clutch of eggs; or (2) in the case of multiparasitism, exposing a host recently parasitised by a female of one species to a female of another species.

In both cases, the time taken from oviposition to adult eclosion and the size or weight of emerging adults need to be measured and the different treatments (i.e., initial and second clutches of different sizes) compared with one another and with controls. With ectoparasitoids, eggs can be artificially added to existing clutches of various sizes (Sect. 1.10, and Strand & Godfray, 1989). Assuming competitive equivalence of clutches produced by different females, the effects upon parasitoid growth and development of simultaneous oviposition by two conspecific females would be analogous to the effects of increasing the primary clutch. That is, an increase in the number of eggs laid per host would have a negative effect, irrespective of whether the eggs are laid by one or by different females, provided all the eggs are laid at the same time. However, the competitive disadvantage of a second clutch may be underestimated from a fitness function curve that is based solely on initial clutches, if there is a significant time interval between the laying of initial and subsequent clutches (Strand & Godfray, 1989). Measurement of any such disadvantage, in terms of growth and development, to a second clutch requires the progeny from the two clutches to be distinguishable by the investigator. This is possible in those species in which there are mutant strains, e.g., the eye/body colour mutant 'cantelope-honey' in *Habrobracon hebetor*. To ensure, when using mutants, that competitive asymmetries do not bias the results of experiments, reciprocal experiments should be carried out for each clutch size and time interval combination (Strand & Godfray, 1989). Molecular markers can also be used (Sect. 3.2.2).

The possibly complicating effects of sex differences in larval food acquisition also need to be borne in mind in experiments on gregarious parasitoids: compared with the adding of a female egg, the adding of a male egg to an existing clutch could have less of an effect upon fitness of the progeny in the initial clutch.

2.9.3 Effects of Physical Factors on Growth and Development

2.9.3.1 Temperature

The importance of understanding the effects of temperature extremes on the growth, development, survival and reproduction of insects is becoming increasingly recognised in a warming world where climate extremes and heat waves are increasing in their frequency, duration and intensity (Perkins et al., 2012; Perkins-Kirkpatrick and Lewis, 2020; Sect. 2.7.4). Recent reviews highlight the effects of heat exposure on insect physiology and ecology (González-Tokman et al., 2020; Harvey et al., 2020; Ma et al., 2021).

Development Rate

Figure 2.52 shows the typical relationship between an insect's rate of development and temperature. There is a threshold temperature below which there is no (measurable) development; this threshold is sometimes referred to as the

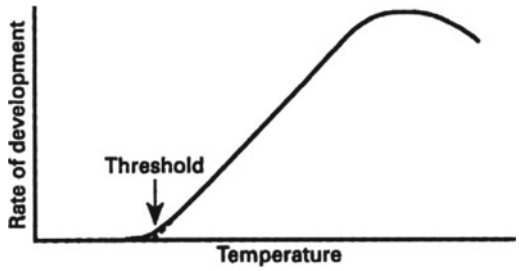


Fig. 2.52 The rate of insect development as a function of temperature. *Source* Gilbert et al. (1976). Reproduced by permission of W.H. Freeman & Co

developmental zero. There is also an upper threshold above which further increases in temperature result in only small increases in development rate. The overall relationship is non-linear (Mills, 1981), but over the intermediate range of temperatures normally experienced by an insect species in the field, it is linear. As noted by Gilbert et al. (1976), why this should be so is a mystery, since rates of enzyme action (which are presumably basic to development) usually increase exponentially, not linearly, with increasing temperature.

The deleterious effects of a high temperature extreme depend on how long the insect is exposed to it. As pointed out by Campbell et al. (1974) with reference to the development rate–temperature relationship shown in Fig. 2.52, temperatures within the high range (i.e., the part of the relationship where the curve decelerates) have a deleterious effect upon development only if the temperature is either held constant within the range or fluctuates about an average value within the range. If the temperature fluctuates about a daily average within the medium range (i.e., the linear part of the relationship) and the daily maximum reaches the high range, no deleterious temperature effect is observed.

Given the fact that the development rate *versus* temperature relationship is linear over the greater range of temperatures, the total amount of development that takes place during any given time period will be proportional to the length of time multiplied by the temperature above the threshold. With this physiological time-scale of day-degrees development proceeds at a constant rate, whatever the actual temperature. This concept is elaborated upon below.

To study the dependency of overall development rate on temperature in a parasitoid, expose hosts to female parasitoids at different constant temperatures (the range being chosen on the basis of field temperature records), and measure the time taken from oviposition to adult eclosion. To demonstrate the effect of temperature on overall larval development in a predator, provide different cohorts of larval predators with a fixed daily ration of prey, at different temperatures, from egg hatch to adult eclosion. With both parasitoids and predators the thermal requirements for development can be determined for particular stages, i.e., the egg (Frazer & McGregor, 1992, on coccinellids), each larval instar and the pupa.

The data obtained from the above experiments can be described by a linear regression equation of the form:

$$y = a + bT \tag{2.16}$$

where y is the rate of development at temperature T, and a and b are constants. If the regression line were to be extrapolated back, it would meet the abscissa (x-axis) at the developmental zero, t, which may be calculated from $t = -a/b$. The total quantity of thermal energy required to complete development, the thermal constant (K) can be calculated from the reciprocal of the slope of the regression line, $1/b$.

Once t and K have been calculated from data obtained at constant temperatures, the rate of development under any fluctuating temperature regime can be determined by thermal summation procedures. Unit time-degrees (day-degrees or hour-degrees) above t are accumulated until the value of K is reached where development is complete. This can be done either by accumulating the mean daily temperature minus the lower threshold or by accumulating the averages of the maximum and minimum daily temperature minus the threshold (i.e., $\sum[\{(T_{\max} - T_{\min})/2\} - \text{threshold}]$). However, both of these methods will result in great inaccuracies if a temperature contributing to the mean lies outside of the linear portion of the

relationship. Means, by themselves, give no indication of the duration of a temperature extreme: an apparent tolerable mean temperature may actually comprise a cyclical regime of two extremes at which no development is possible. A much more accurate method is to use hourly mean temperatures (Tingle & Copland, 1988).

Summation has been used by many workers, including Butts and McEwen (1981), Osborne (1982), Goodenough et al. (1983), Nealis et al. (1984), Cave and Gaylor (1988), Rodriguez-Saona and Miller (1999) and Bazzocchi et al. (2003), Chong and Oetting (2006), Pandey and Tripathi (2008), Papanikolaou et al. (2013) and Honek et al. (2018). However, the method has been much criticised as it has two inherent faults. First, the assumed linear relationship is known to hold as an approximation for the median temperature range only (Fig. 2.52) (e.g., Campbell et al., 1974, on aphid parasitoids; Syrett & Penman, 1981, on lacewings). Second, the lower threshold upon which summation is based is a purely theoretical point determined by extrapolation of the linear portion of the relationship into a region where the relationship is unlikely to be linear. The linear model is likely to underestimate development rates when average daily temperatures remain close to the threshold for long periods, although this can easily be corrected for (Nealis et al., 1984). In an attempt to improve upon the thermal summation method, an algorithm was developed using a sigmoid function with the relationship inverted when the temperature is above the optimum (Stinner et al., 1974). The assumed symmetry about the optimum is unrealistic, but Stinner et al. (1974) argue that the resultant errors are negligible. This algorithm has also been used in simulations for *Encarsia perniciosi* (McClain et al., 1990), fly parasitoids (Ables et al., 1976) and other insects (Berry et al., 1976; Whalon & Smilowitz, 1979; Allsopp, 1981). Ryoo et al. (1991) used a combination model involving upper thresholds to describe the development of the ectoparasitoid *Lariophagus distinguendus* (Pteromalidae).

In some cases, the improvement in accuracy of simulations over the thermal summation method has been small or negligible and it is questionable whether the use of complex models is necessary in relation to normal field conditions (Kitching, 1977; Whalon & Smilowitz, 1979; Allsopp, 1981). The method of matched asymptotic expansions was used to develop an analytical model describing a sigmoidal curve that lacks the symmetry about the optimum found in the algorithm of Stinner et al. (1974). Again, the authors concerned claimed excellent results (Logan et al., 1976). However, comparisons of linear and non-linear methods to validate field data for *Encarsia perniciosi* showed no great differences (McClain et al., 1990).

Other non-linear descriptions of the development rate–temperature relationship have also been developed. These include the logistic curve (Davidson, 1944) and polynomial regression analysis (Fletcher & Kapatos, 1983). Polynomial regression analysis can be used to select the best-fitting curve to a given set of data. Successively higher-order polynomials can be fitted until no significant improvement in F-value results. This approach was found useful in describing data for *Diglyphus intermedius* (Patel & Schuster, 1983) and mealybug parasitoids (Tingle & Copland, 1988; Herrera et al., 1989). Higher-order polynomials may produce unlikely relationships between data points and fluctuate widely outside them. Before selecting a particular fit, it should be examined over the entire range of the data. It may be better to choose one that has a comparatively poor fit but is biologically more realistic (Tingle & Copland, 1988).

Several authors have reported acceleration or retardation of development, when comparisons are made between development periods at cycling temperatures and at a constant temperature equivalent to the average of the cycling regime. The question of whether these effects are an artefact or are a real biological phenomenon is discussed by Tingle and Copland (1988).

Until recently, data on the development times of insects were almost always expressed in the form of means and standard deviations (Howe, 1967; Eubank et al., 1973; Sharpe et al., 1977). Several models have been developed which include a function to account for the asymmetrical distribution of development times (Stinner

et al., 1975; Sharpe et al., 1977; Wagner et al., 1984). Such models can be incorporated into population models (Barfield et al., 1977b, on *Habrobracon mellitor*). However, the poikilotherm model of Sharpe et al. (1977) did not give any great improvement in accuracy over day-degree models when predicting development of *Trichogramma pretiosum* (Goodenough et al., 1983).

Biological control workers can use laboratory-obtained information on the effects of temperature on development in deciding which of several candidate species, 'strains' or 'biotypes' of parasitoids and predators to either introduce into an area or use in the glasshouse environment. In classical biological control programmes, the usual practice is to introduce natural enemies from areas having a climate as similar as possible to that in the proposed release area (Messenger, 1970; Messenger and van den Bosch, 1971; van Lenteren, 1986; Sect. 7.4.3). If there are several species, strains or biotypes to choose from, the one found to have a temperature optimum for development that is nearest to conditions in the introduction area should be favoured, all other things being equal.

A classic example of a biological control failure resulting from the agent being poorly adapted to the climate of the introduction area is the introduction of a French strain of *Trioxys pallidus* into California to control the walnut aphid. This parasitoid was poorly adapted to conditions in northern and especially central California where it never became permanently established. The French strain was unable to reproduce and survive to a sufficient extent in areas of extreme summer heat and low humidity. A strain from Iran was subsequently introduced and proved far more effective (DeBach & Rosen, 1991).

Data on development rate–temperature relationships are used in population models to investigate dynamics and phenologies in a biological control context (Chap. 7). Morales and Hower (1981) showed that they could predict the emergence in the field of 50% of the first and second generations of the weevil parasitoid *Microctonus aethiopoides* (Braconidae) by using the day-degree method. Goldson et al. (1998) applied a phenological model retrospectively to the phenology of *Microctonus hyperodae* and its weevil host. McClain et al. (1990) used the linear day-degree model and the sigmoid function model of Stinner et al. (1974) to predict the peaks of activity of parasitoids in orchards. The linear model predicted 8 of 13 peaks within ±7 days, while the non-linear model was accurate for 7 of 13 peaks. Horne and Horne (1991) showed that simple day-degree models could account for the synchronisation of emergence of the encyrtid parasitoid *Copidosoma koehleri* and its lepidopteran host.

Growth Rate

Most studies on temperature relationships have dealt with development but have ignored growth. The relationship between growth rate and temperature in insects has been shown by direct measurement to increase linearly with temperature within the range of temperatures normally experienced by the insect in the field, in accordance with the following model:

$$\frac{1}{w}\frac{dw}{dt} = a(T - \theta) \qquad (2.17)$$

where T is the temperature, θ is the threshold temperature below which no growth occurs, w is the larva's weight at time t, and a is a constant (Gilbert, 1984). Gilbert (1984) used this model to predict pupal weight, which determines fecundity, in the butterfly *Pieris rapae*. Tokeshi (1985) describes another method for estimating minimum threshold temperature and day-degrees required to complete growth, suitable for use with aquatic or terrestrial insects in either the laboratory or the field.

In some predator species the tendency is for successive larval instars to achieve a growth rate maximum at a higher temperature, e.g., in *Adalia bipunctata* the maxima recorded were 20°, 22.5°, 22.5° and 25 °C for the first, second, third and fourth instars respectively (Mills, 1981). Mills (1981) suggested these differing optima could reflect the increasing temperatures experienced

by the coccinellid larvae as they progress through the life-cycle in the field. Aksit et al. (2007) found a linear, negative relationship between temperature and rate of development of immature (eggs and larvae) in the mite-feeding ladybird *Stethorus gilvifrons*. However, in other predators, there is no such tendency, e.g., in the damselflies *Lestes sponsa*, *Coenagrion puella* and *Ischnura elegans* maximum development rates were recorded at the same temperature for the last five instars (Pickup & Thompson, 1990).

Interaction Between Temperature and Consumption Rate

Whilst temperature will affect growth and development rates of predators directly, one has to be aware that it can also exert an influence by changing the prey consumption rate (Mills, 1981; Gresens et al., 1982; Sopp & Wratten, 1986; Pickup & Thompson, 1990; Fig. 2.53). The rate at which food passes through the gut will be positively temperature dependent, and this will affect consumption rate by affecting hunger (insect hunger is directly related to the degree of emptiness of the gut; Johnson et al., 1975). *B* in Eqs. 2.10–2.15 (representing in part basal metabolic costs) will also be temperature dependent (Pickup & Thompson, 1990), and consumption rate will increase to counteract an increase in *B*.

To take any confounding effects of varying consumption rate into account when assessing the influence of temperature on growth and development rates in *Adalia bipunctata*, Mills (1981) compared the mean growth and development rates recorded at the experimental range of temperatures (i.e., fixed daily ration of prey) with those predicted from Fig. 2.41 (i.e., constant temperature regime) for the appropriate rates of consumption (Fig. 2.54). With this analysis, Mills (1981) recorded significant deviations from the predicted growth and development rates, indicating that temperature does have a direct influence on growth and development.

A more straightforward approach to determining how consumption rate interacts with

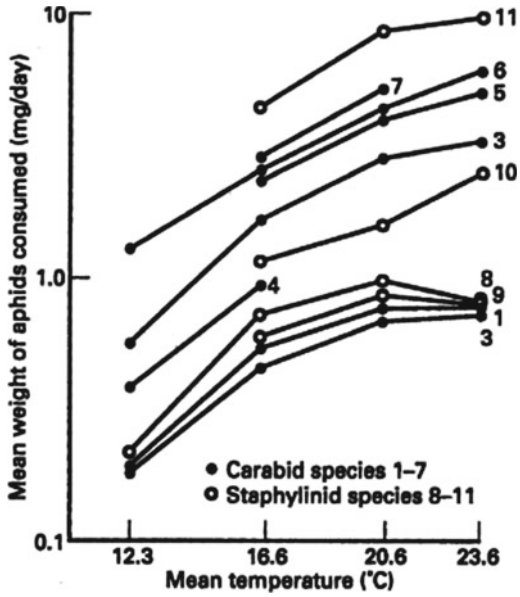


Fig. 2.53 The effect of temperature on the mean weight of aphids (*Sitobion avenae*) consumed per day by eleven species of carabid and staphylinid beetles. In the experiments, individual beetles were given an excess of prey (first and second instar, in approximately equal proportions). *Source* Sopp and Wratten (1986)

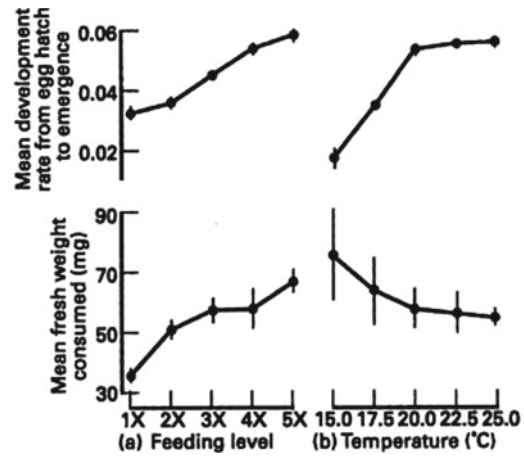


Fig. 2.54 The mean (± SE, $n = 6$–10) rates of prey consumption and development of the immature stages of *Adalia bipunctata* (Coccinellidae) in relation to prey availability at: **a** a constant temperature (20 °C) and various 'feeding levels' (the weight of prey corresponding to 1 to 5 times the average teneral weight of the instar); **b** a range of temperatures, using one (4 times) feeding level. *Source* Mills (1981). Reproduced by permission of Blackwell Publishing

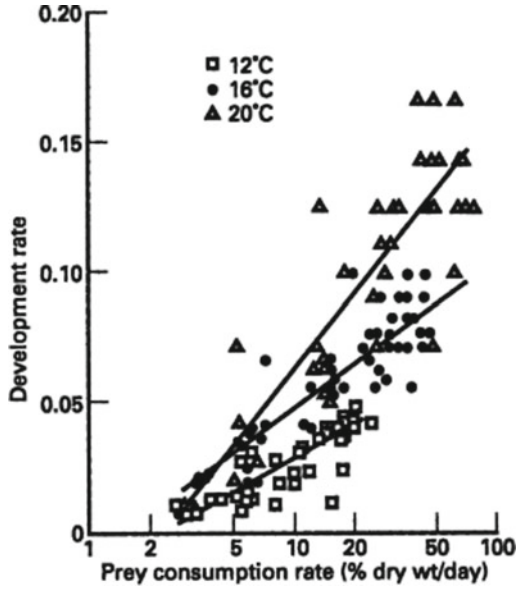


Fig. 2.55 Development rate in relation to consumption rate (note log scale) in *Coenagrion puella* (Odonata: Zygoptera). Temperature affects development directly and indirectly by increasing the prey consumption rate. There is a clear interaction effect between temperature and consumption rate. *Source* Pickup and Thompson (1990). Reproduced by permission of Blackwell Publishing

temperature to affect development rate involves plotting development rate against consumption rate, constructing regression lines for each temperature regime and then comparing the slopes of the lines. As can be seen from plots for the damselfly *Coenagrion puella* (Fig. 2.55), higher consumption rates produce stronger developmental responses to increases in temperature.

2.9.3.2 Other Physical Factors

Diurnal predator larvae may, like the adults (Sect. 2.7.4), show a reduction in daily consumption rate with decreasing photoperiod, and this will be reflected in a reduction in growth and development rates. Bear in mind, when varying photoperiod in experiments, that you may also be inadvertently varying the absorption of radiant energy by insects, thus altering their body temperature.

Larvae of terrestrial predators may, like the adults, increase their rate of prey consumption with decreasing humidity, which will cause them to grow larger and more rapidly. Predator larvae may develop faster in an incubator than in a large environment chamber, even at the same temperature, because of the lower humidity in the former (Heidari, 1989).

For a study of the effects of photoperiod on parasitoid development, see Urbaneja et al. (2001a).

2.10 Survival of Immatures

2.10.1 Introduction

Below we discuss some factors that affect the survival of predator and parasitoid immatures. Parasitism and predation by heterospecifics are not considered (see Chap. 7 for practical approaches), whereas predation by conspecifics, i.e., cannibalism (Sabelis, 1992) is. Mortality of parasitoid juveniles is strongly dependent on that suffered by the hosts that support them. Hosts may be killed through predation, starvation and exposure to unfavourable weather conditions, and any parasitoids that are attached to or contained within the hosts will die. Price (1975) illustrated this relationship by reference to the host survival curves, which in insects are of either Type II or Type III (Fig. 2.35), i.e., substantial mortality of hosts (very substantial in the latter case), and therefore of any parasitoid progeny they support, occurs by the mid-larval stage (see also Cornell et al., 1998).

When investigating larval mortality, the possibility ought to be considered that some factors may cause higher mortality in one sex than in another. Some parasitoids allocate male eggs to small host individuals and female eggs to large individuals (Sect. 1.11). If small hosts suffer a higher degree of mortality from a predator than larger ones, then the survival rates of male and female parasitoids will differ.

2.10.2 Effects of Biotic Factors on Survival of Immatures

2.10.2.1 Food Consumption by Predators

By recording deaths of individuals within each instar in the food consumption experiment outlined earlier, the relationship between food consumption and survival can be studied. A model relating larval survival to prey availability was developed by Beddington et al. (1976). If we assume that we are not dealing with a population of genetically identical individuals, then we would expect mortality through food shortage to take place at some characteristic mean ingestion rate μ_i, with the population as a whole displaying variation about this mean value. Assuming that the proportion of the population experiencing 'food stress' is normally distributed about the mean, with standard deviation σ, then the proportion (S) of the larval population surviving to complete development within any particular instar of duration d, at an ingestion rate I, will be given by (Beddington et al., 1976):

$$S = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{z} \exp\left(-\frac{z^2}{2}\right) dz \qquad (2.18)$$

where $z = \frac{I-\mu_i}{\sigma_i}$.

Using Eqs. 2.2 and 2.18, S may be expressed in terms of either consumption rate or prey density (Fig. 2.56). The relationship in Fig. 2.56c is shown by predators in the laboratory (Fig. 2.57). As pointed out by Beddington et al. (1976), whether a survival curve rises extremely rapidly or slowly depends on the range of prey densities over which experiments are carried out and the graphical scales chosen for plotting the data.

Mortality due to nutritional stress apparently occurs at feeding rates very much higher than the minimum rate necessary for growth and development, so that individuals that are growing normally (albeit slowly) at low feeding rates are apparently highly likely to suffer high mortality at the moult (Beddington et al., 1976). There may

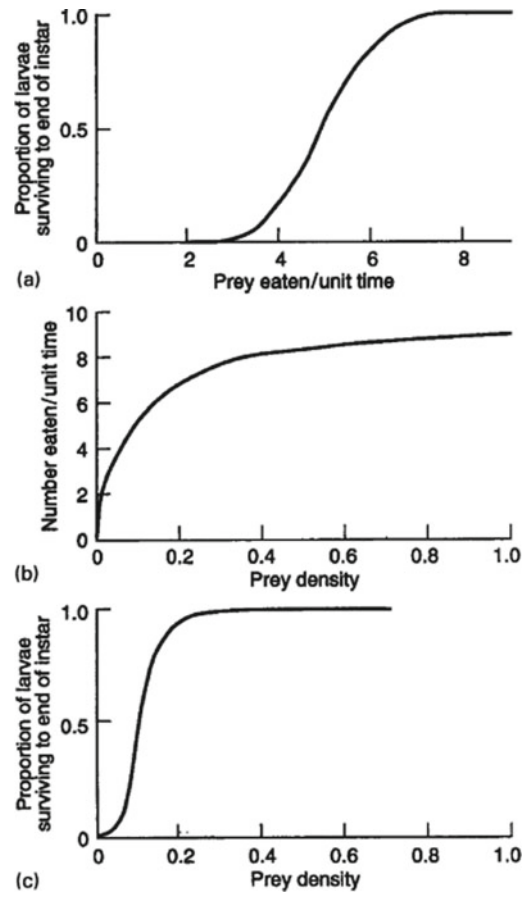


Fig. 2.56 Hypothetical relationships between **a** the proportion of individual predators surviving to the end of an instar and their mean feeding rate during that instar; **b** predation rate and prey density; **c** the relationship obtained by combining **a** with **b**. *Source* Beddington et al. (1976). Reproduced by permission of Blackwell Publishing

be no relationship between the overall survival rate between entering and leaving an instar (S) and the duration of each instar (d), as in *Blepharidopterus angulatus*, or S may decline in a variety of ways with increasing d (examples are given in Beddington et al., 1976).

Survival rates vary between successive instars at comparable prey densities. Figure 2.58 summarises the relationship between instar and the feeding rate at which 50% of a larval cohort survive, in four predatory insects and a spider. The plots indicate a constant increase in feeding

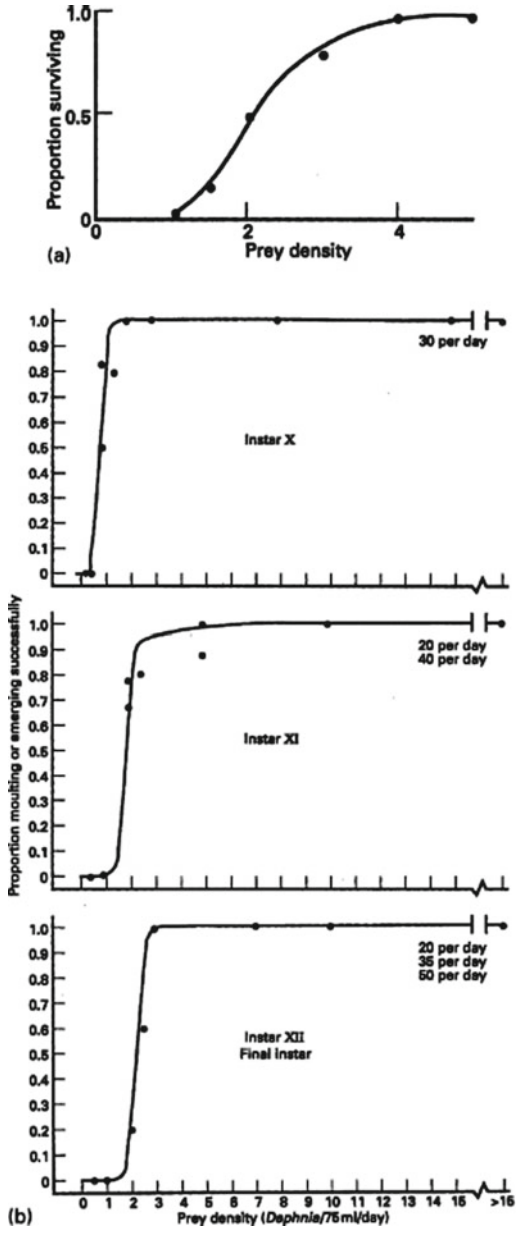


Fig. 2.57 The relationship between the proportion of predators surviving to the end of an instar, and the mean density of prey available during that instar. **a** First instars of the coccinellid beetle *Adalia bipunctata* (data from Wratten, 1973); **b** tenth, eleventh and twelfth (final) instars of the damselfly *Ischnura elegans* (*source* Lawton *et. al*., 1980). Reproduced by permission of Blackwell Publishing

rate between instars to maintain survival rates at 50%. In the case of *Ischnura elegans*, feeding rates necessary to ensure 50% survival approximately doubled between instars ten and eleven, and increased by a factor of 1.4 between instars eleven and twelve (Lawton et al., 1980). The theoretical line in Fig. 2.58 is for larvae that double their body weight between instars and in which minimum requirements increase by a 0.87 power of body weight (which they do for larvae of a damselfly closely related to *Ischnura*). The steeper slopes shown by *Adalia*, *Notonecta* and the spider perhaps reflect the higher exponents in their metabolic rate:weight relationships and/or larger increases in average body weights between instars (Lawton et al., 1980).

2.10.2.2 Prey Species

The relationship between larval survival and prey species has been most thoroughly investigated in coccinellid beetles (Hemptinne et al., 2000; Wiebe & Obrycki, 2002; Özder & Sağlam, 2003; Barbosa et al., 2014). Coccinellid larvae have been presented with acceptable prey of various species, and larval mortality measured (Hemptinne et al., 2000, studied intraguild predation). In the lacewing, *Dichochrysa prasina*, preimaginal survival, development time, adult longevity and fecundity were significantly affected when fed on different kinds of prey, such as the eggs, larvae and/or adults of aphids, moths and beetles (Pappas et al., 2007).

Consumption rate in relation to each prey species can be measured, and prey-related differences in survival correlated with differences in consumption rate. However, if there is a reduced rate of consumption on a prey species and survival is also low on that species, one cannot necessarily conclude that poor survival is a direct result of reduced consumption rate. Survival on the 'better' prey species may still be higher than on the 'poorer' one at equivalent consumption rates (Hodek, 1973). If it is, then the prey-related difference in survival may be due to factors such

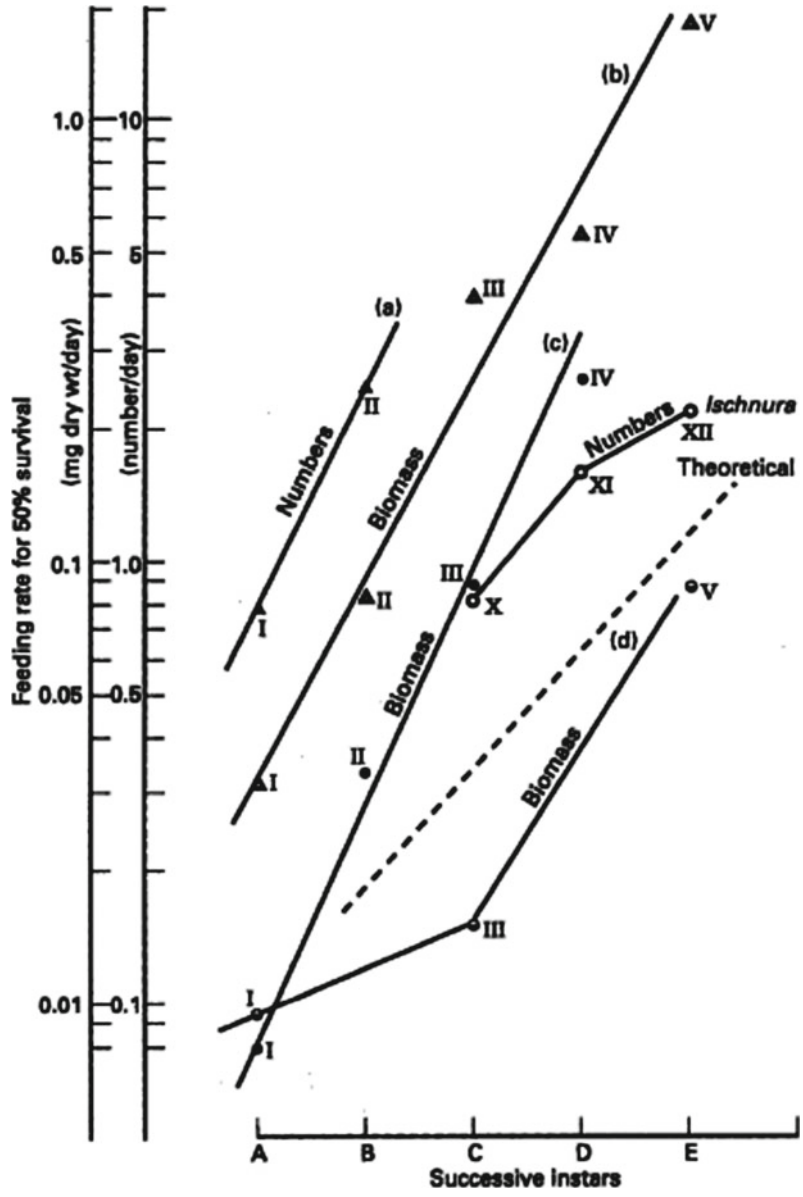


Fig. 2.58 The relationship between instar number (in Roman numerals) and the consumption rate (feeding rate) at which 50% of a cohort of larvae in a particular instar successfully complete their development (LD_{50} for food stress): **a** *Adalia decempunctata* (Coccinellidae) (data from Dixon, 1959); **b** *Notonecta glauca* (Notonectidae) (data from McArdle, 1977); **c** *Linyphia triangularis* (Arachnida: Linyphiidae) (data from Turnbull, 1962); **d** *Blepharidopterus angulatus* (Miridae) (data from Glen, 1973). The theoretical line is for larvae that double their body weight between instars, and where food-energy requirements increase by a 0.87 power of body weight. *Source* Lawton et al. (1980). Reproduced by permission of Blackwell Publishing

as differences in the size or qualitative attributes of the prey species.

2.10.2.3 Interference and Exploitation Competition, Cannibalism

Interference from conspecifics and predators can, through its effects on feeding rates, potentially reduce survival (Heads, 1986; Sih, 1982, Sect. 2.9.2). Exploitation is an obvious potential cause of mortality among larval conspecifics, as prey may be depleted to a level at which larvae experience nutritional stress.

Cannibalistic behaviour is an additional cause of mortality in the immature stages of dragonflies, damselflies, water-boatmen, coccinellid beetles, ground beetles, anthocorid bugs and antlions (Mills, 1982; Crowley et al., 1987; Sih, 1987; Nasser & Abdurahman, 1990; Agarwala & Dixon, 1992; Griffiths, 1992; Hopper et al.,

1996; Gagné et al., 2002; Michaud, 2003; Frank et al., 2010). The adults and larvae of several Coccinellidae are known to be cannibalistic on eggs (Gagné et al., 2002) [The neonate larvae of the coccinellid *Coleomegilla maculata lengi* prefer the eggs of conspecifics to aphid prey (Gagné et al., 2002).] In dragonflies, cannibalism may result in the death not only of one of the interacting pair (same or smaller instar larva) but also of both participants, since it could attract the attention of predators (Crowley et al., 1987) (this also applies to non-cannibalistic interference). Hopper et al. (1996) showed that in the dragonfly *Epitheca cynosura* cannibalism among larvae was more important than exploitation competition in determining survival; they also found that when juveniles hatch asynchronously in close proximity, cannibalism is density dependent (so can therefore contribute to population regulation), and they concluded that it can also increase population synchrony by exerting size-specific mortality on smaller individuals throughout development.

The effects of competition or cannibalism on survival in immature stages can be expressed as either percentage mortality plotted against predator density or as *k*-values for the mortality plotted against $\log_{10}$ predator density (Varley et al., 1973; Sect. 7.3.4). If density-dependent mortality occurs, it will be shown within the upper range of densities only, i.e., there will be a threshold density of predators below which *k* is zero (Mills, 1982) (or its value is slighty above zero, in which case one has to question whether the mortality recorded at low predator densities is entirely attributable to interference, exploitation or cannibalism). The manner in which *k* varies with $\log_{10}$ predator density indicates the nature of the density dependence, i.e., exact, over- or under-compensation (Sect. 7.3.4) and whether competition is of the scramble type or contest type (for explanations of these terms, see Varley et al., 1973; Begon et al., 1996).

Bear in mind that for the perpetrator, survival may be improved by cannibalism: larvae of the coccinellid *Cycloneda sanguinea* had a higher survival rate when fed conspecific eggs, than when fed moth eggs (Michaud, 2003).

Destructive host-feeding by parasitoids will very rapidly kill any parasitoid immatures contained within the host (Jervis & Kidd, 1986; Kidd & Jervis, 1991). Non-destructive host-feeding is unlikely to kill parasitoid immatures in the short-term, but could nevertheless reduce their life expectancy (e.g., Heimpel & Collier, 1996; Ueno, 1997).

2.10.2.4 Host Size, Age or Stage

As well as measuring growth and development of parasitoids, larval survival can also be recorded in relation to host size at oviposition. One might reasonably assume, for nutritional reasons, that generally for solitary idiobionts survival is highest in large hosts, although there could be cases where hosts above a certain size represent a resource in excess of the amount required by the larva to complete its development (in such cases larval survival may not be improved in the largest hosts, and it may even be reduced, e.g., due to putrefaction of the remaining host tissues, see also Ode & Strand, 1995) (Sect. 2.9.2).

The relationship for koinobionts is likely to be more complex. For the solitary koinobionts *Lixophaga diatraeae* and *Encarsia formosa*, survival is highest in individuals that complete their development in medium-sized hosts (Miles & King, 1975; Nechols & Tauber, 1977). For the solitary koinobiont *Leptomastix dactylopii*, no significant differences were found between survival in different-sized hosts (de Jong & van Alphen, 1989). In *Venturia canescens* survival is highest in medium-sized hosts and lowest when the second-instar host is oviposited in (Fig. 2.47c). The probable reason for the lower survival in second-instar hosts is injury to the host through insertion and removal of the ovipositor (this does not occur when later instars are attacked) (Harvey et al., 1994). By contrast, in the solitary endoparasitoid *Microplitis demolitor*, survival was lowest in 1-day old (early L1) hosts and 6–8-day-old (late L3 and L4) hosts (*Chrysodeixis includens* caterpillars) and highest in 2–4-day-old (late L1 and L2) hosts (Harvey et al., 2004). In this association, encapsulation of parasitoid eggs and larvae in larger hosts probably accounted for higher mortality.

However, some fully developed parasitoid larvae were unable to egress from the host, presumably because they were unable to perforate the cuticle with their mandibles. Unlike larvae of *V. canescens*, which consume virtually the entire host before pupation, larvae of *M. demolitor* are haemolymph-feeders and thus consume only a relatively small fraction of tissues of larger hosts (Harvey, 2005; Harvey & Malcicka, 2016).

As described above, a possible complicating factor in experiments is mortality from encapsulation, which may be higher in some stages than in others (usually larger, later-instar hosts possess stronger immune defences than young larvae). Therefore, samples of hosts need to be taken and dissected during larval development to provide data on the frequency of encapsulation, so that this potential confounding factor can be controlled for in data analyses.

In those gregarious parasitoids in which progeny survival is 100% at the smallest clutch size, 100% survival might also occur at larger clutches if larger-sized hosts are utilised. The slope of the relationship might also be less steep in the case of larger hosts.

2.10.2.5 Host Age

Host age, rather than host size, could influence parasitoid survival. The effects of the two variables may, however, be difficult to disentangle. Survival in some egg parasitoids depends on the age at which the host egg is attacked (Ruberson & Kring, 1993).

2.10.2.6 Host Species

Given that different host species are likely to constitute different resources, both qualitatively and quantitatively, parasitoid larval survival may vary with host species, the larvae (or eggs) dying through malnutrition, encapsulation (see Host Physiological Defence Reactions, below) or poisoning (e.g., if the host has sequestered toxins from its food plant, see Multitrophic Interactions and The Performance of Immatures, above).

In *Telenomus lobatus* percentage eclosion, i.e., survival of progeny, was higher from the eggs of *Chrysoperla* species than from eggs of *Chrysopa* species (Ruberson et al., 1989). In the egg parasitoid *Trissolcus basalis* oviposition in eggs of the Brown Marmorated Stink Bug, *Halyomorpha halys*, an invasive pest, rather than of the normal host (the Southern Green Stink Bug, *Nezara viridula*), leads to considerably lower offspring survival, and has been seen as an 'evolutionary trap'; however, the few survivors can be unusually large and this may suffice to offset the disadvantage (Mesterton-Gibbons et al., 2021, and references therein). In the gregarious idiobiont *Habrobracon hebetor*, survival within clutches was density dependent both on a small moth species, *Plodia interpunctella*, and on a large moth species, *Anagasta kuehniella*, but the density dependence in the latter case applied only to very high (artificially manipulated) clutch sizes (Taylor, 1988).

To investigate the effect of host species on larval survival, present females with hosts of different species and, if possible, of equivalent size and age. Maintain the hosts until the parasitoids pupate, and maintain the parasitoid pupae until the adults have ceased emerging. Any hosts that have received eggs but have not given rise to parasitoids should be examined (dissected in the case of endoparasitoids) for the remains of parasitoid eggs or larvae. Any parasitoid pupae that fail to produce adults should also be recorded. Sex differences in survival should also be established (Ruberson et al., 1989).

2.10.2.7 Hosts' Food Plant

See the subsection Multitrophic Interactions and the Performance of Natural Enemies (Sect. 2.9.2.9).

2.10.2.8 Superparasitism and Multiparasitism

Solitary Parasitoids

In solitary endoparasitoids, supernumerary larvae are eliminated (contest competition) either through physiological suppression or (more usually) through combat (Clausen, 1940; Fisher, 1971; Vinson & Iwantsch, 1980b; Quicke, 1997; Harvey et al., 2013a, b). This applies to self- and conspecific superparasitism as well as heterospecific superparasitism (= multiparasitism).

The first-instar larvae of almost all solitary parasitoid wasp species are equipped with robust, often sickle-shaped mandibles (Fisher, 1961; Salt, 1961; Fig. 2.59). Fighting often takes place between larvae that are of approximately the same age, although in some species first-instar larvae will attack and kill later instars that either have reduced mandibles or lack mandibles altogether (Chow & Mackauer, 1984, 1986). Note that the possession by first-instar larvae of large mandibles does not necessarily mean that fighting is the sole mechanism employed in the elimination of rivals (Strand, 1986; Mackauer, 1990). Also, bear in mind that the first-instar larvae of some facultatively gregarious species possess sharp mandibles, but the larvae do not practise siblicide (e.g., *Aphaereta pallipes*, Mayhew & van Alphen, 1999).

The mechanisms employed in the elimination of larval competitors in three solitary braconid parasitoids are summarised in Fig. 2.60. As with other parasitoids, in cases of intraspecific larval competition the oldest larva generally survives and the younger larva dies, although this may not apply where the larval age difference is either very small or very large (in the latter case the older larva may have developed to the second, i.e., non-mandibulate instar by the time the second egg is either laid or hatches; Bakker et al., 1985; see also Marris & Casperd, 1996).

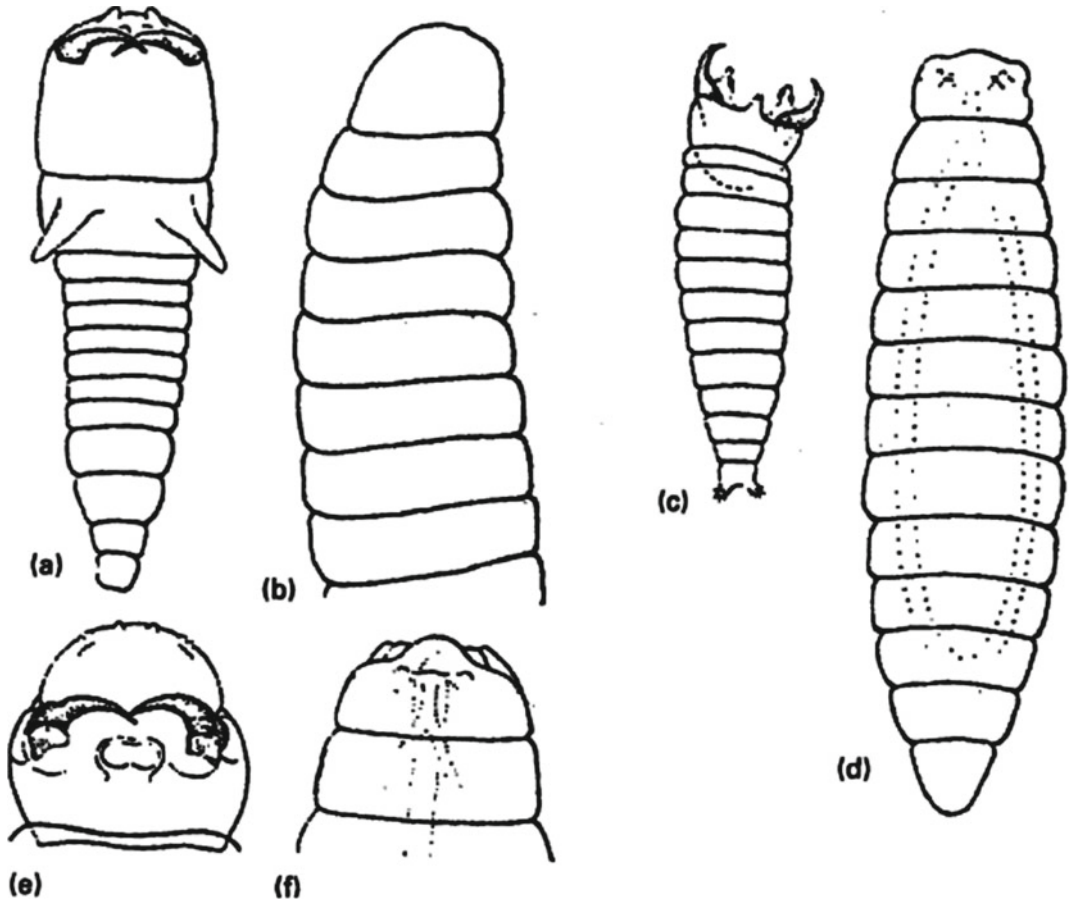


Fig. 2.59 Larvae of parasitoid wasps that in the first instar have mandibles for fighting (**a**, **c**, **e**) but do not have such mandibles in the second instar (**b**, **d**, **f**). **a**, **b** *Biosteres fletcheri* (Braconidae); **c**, **d** *Psilus silvestri* (Diapriidae). **e**, **f** *Diplazon fissorius* (Ichneumonidae). *Source* Salt (1961), reproduced by permission of The Company of Biologists Ltd

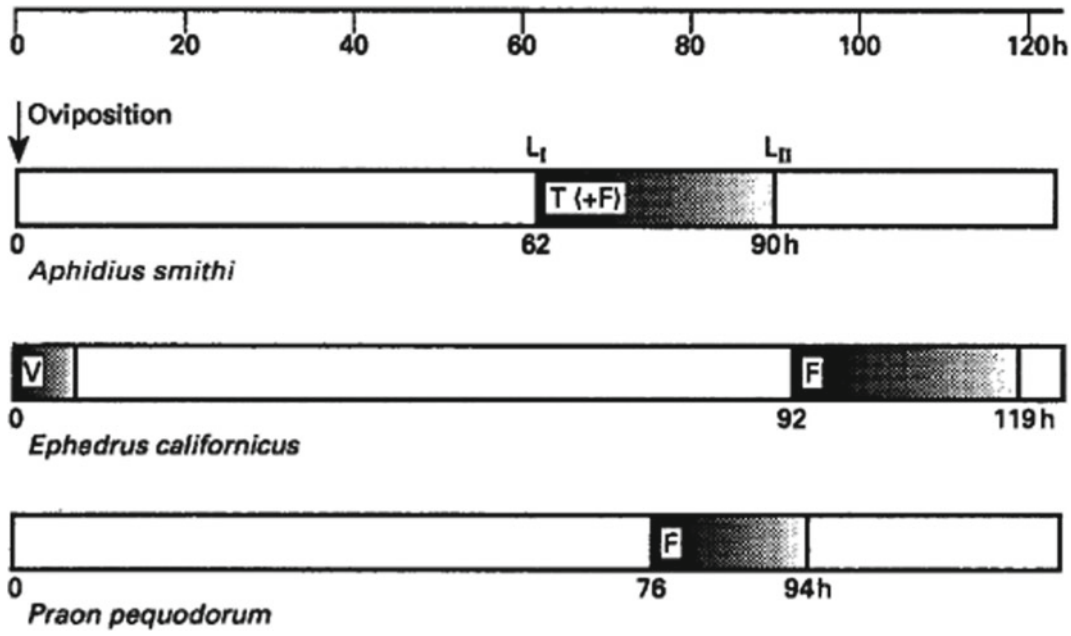


Fig. 2.60 The mechanisms used in the elimination of competitors by the solitary braconid parasitoids *Aphidius smithi*, *Ephedrus californicus* and *Praon pequodorum* in pea aphids. F = fighting among first-instar larvae (L_I); T = toxin released at eclosion of L_I; V = venom injected by female at oviposition. Median times taken from eclosion to L_I and L_{II} refer to parasitoid larvae developing in second-instar pea aphids at 20 °C. *Source* Mackauer (1990). Reproduced by permission of Intercept Ltd

The 'oldest larva advantage' applies to some cases of interspecific larval competition among parasitoids but not to others (Mackauer, 1990; Tillman & Powell, 1992; de Moraes et al., 1999; de Moraes & Mescher, 2005; Harvey et al., 2009a, b, 2013a, b; Cingolani et al., 2013; Chen et al., 2019b, see below). Relative age differences can influence the outcome of an interaction, but the factors that appear to be more important in determining who survives are the particular competitive mechanism(s) and the developmental stage at which each comes into play. Bear in mind that: the eggs of two species may be laid at the same time, but hatch at different times, and/or the development rate of the larva may be greater in one species than in another, and these factors may determine the 'window of interaction'. For example, the braconids *Aphidius smithi* and *Praon pequodorum* require approximately the same amount of time to develop from oviposition to the second instar, but the embryonic period is much shorter in *Aphidius* than in *Praon*. This difference enables *Praon* to compete as a mandibulate first-instar larva with an older *Aphidius* larva. *Aphidius* larvae usually survive only if they have reached the end of the fourth (final) instar while *Praon* is still in the embryonic stage and thus unable to attack an older competitor (Chow & Mackauer, 1984, 1985).

A parasitoid species that wins under most conditions is described as intrinsically superior (Zwölfer, 1971, 1979). Ectoparasitoids tend to be intrinsically superior to endoparasitoids (Petters & Stefanelli, 1983; Harvey et al., 2013a, b), but see Sullivan (1971) for one exception. The superiority of ectoparasitoids is a result of envenomation and/or more rapid destruction of the host, rather than a result of the endoparasitoid being attacked directly (Askew, 1971; Vinson & Iwantsch, 1980b).

Collier et al. (2002) tested the hypothesis that relative egg size can be used to predict the outcome of 'intrinsic competition' between closely related parasitoid species (*Encarsia* spp.): a

species with relatively large eggs should be superior to one with small eggs. The hypothesis was not supported by the experimental evidence: the species with the smaller eggs (*E. formosa*) prevailed in competition, irrespective of the order of exposure (however, *E. formosa* females killed the progeny of its superior larval competitor by host-feeding).

An experiment designed to investigate the relative competitive superiority of solitary endoparasitoid larvae in instances of superparasitism would involve varying the time interval between ovipositions (from a few seconds to many hours), either by the same parasitoid species or females of different species. If heterospecific superparasitism is being studied, then the sequence of species ovipositions can be reversed. Whatever the type of interaction being investigated, by taking regular samples of the superparasitised hosts and singly parasitised hosts at successive points in time from the second oviposition and dissecting them, the following can be recorded:

1. The stage of development (embryonic or larval) already reached by the older parasitoid at the time of the second oviposition (determine this from dissection of singly parasitised hosts);
2. The stage of development subsequently reached;
3. The stage of development of the younger parasitoid;
4. Which, if any, of the eggs or larvae are dead or alive (exceptionally, both may be dead, as suggested by the data in Table 2.1);
5. Any behavioural evidence of physical combat;
6. Whether either of the parasitoid immatures bear wounds (the latter may show signs of melanisation [Salt, 1961]).

Threshold time intervals for the different outcomes of competition (if there can be more than one outcome) can then be found. Note that for some interactions, the period of time between oviposition and the development of the host to a certain stage indirectly determines which parasitoid species is the survivor. For example, in the case of *Trieces tricarinatus* and *Triclistus yponomeutae*, this interval determines the extent of development of the parasitoids after host pupation and the extent of development at the time of combat (irrespective of whether the host is singly or multiparasitised, development of larvae beyond the first instar can only take place after host pupation) (Dijkerman & Koenders, 1988).

Instead of dissecting superparasitised hosts, the outcome of competition can be studied by rearing the parasitoids to the adult stage. However, in studies of intraspecific superparasitism, this method requires that distinguishable (preferably morphologically) strains be used. This method would also prove useful for studying intraspecific superparasitism when the interval between ovipositions is so short that it is not possible, through dissection, to distinguish between the progeny of the first female and the progeny of the second female. For example, Visser et al. (1992) measured the pay-off from superparasitism in the solitary parasitoid *Leptopilina heterotoma*. They used two strains of this species: a wild type with black eyes and a mutant with yellow eyes. Hosts parasitised by females of one strain were exposed to females of the other strain and the interval between ovipositions was varied. The sequence of ovipositions was reversed to take account of any competitive asymmetry between strains. The probability of a second female realising an offspring from superparasitism, i.e., the pay-off, was then calculated for each strain.

Harvey et al. (1993) examined whether parasitoid mortality from superparasitism varies with host instar in cases of near-concurrent oviposition by two conspecific females (*Venturia canescens*). Parasitoids were reared from third- and fifth-instar hosts (the moth *Plodia interpunctella*) containing either one, two or four parasitoid eggs. Parasitoid mortality was found to be significantly higher in fifth-instar hosts than in third-instar hosts, but within instars did not vary with egg number (Fig. 2.61). The likely reason for the higher mortality in fifth-instar hosts is that

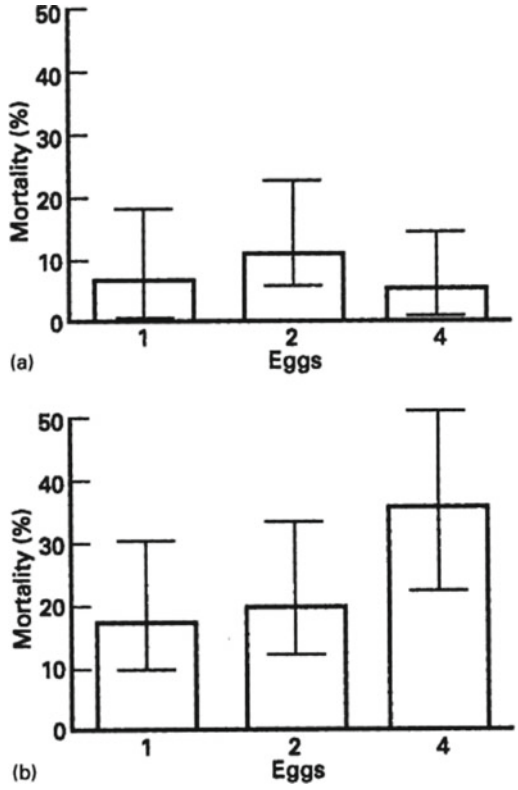


Fig. 2.61 The effects of superparasitism on survival in the solitary ichneumonid parasitoid *Venturia canescens:* Mortality of parasitoids reared from **a** third-instar **b** fifth-instar hosts, containing one, two or four parasitoid eggs. Encapsulation was not a complicating factor in the experiments. Bars show 95% confidence limits for percentages. *Source* Harvey et al. (1993). Reproduced by permission of Blackwell Publishing

there is some physiological incompatibility between the parasitoid and fifth-instar hosts associated with pupation (Harvey et al., 1993).

Gregarious Parasitoids

In gregarious species where survival declines monotonically with increasing clutch size, the addition of an egg or clutch of eggs will (further) reduce percentage survival per host. The reduction will normally result from increased resource competition, since larvae of gregarious species do not engage in physical combat. In those species in which there is an Allee effect (Sect. 1.10), there will be a threshold number of progeny per host below which all parasitoids die, so superparasitism of a host containing a clutch of eggs that is a number short of this threshold number is likely to raise the survival chances of the parasitoid immatures.

Assuming competitive equivalence of first and second clutches laid in or on a host, the effect on survival of simultaneous oviposition by two females would be analogous to the effects of increasing the initial clutch size (Strand & Godfray, 1989). However, in gregarious species mortality may vary not only with the number of eggs initially present but also with the time interval between ovipositions, i.e., it will depend on how soon superparasitism occurs after the laying of the initial clutch (Strand, 1986). Strand and Godfray (1989) demonstrated this for *Habrobracon hebetor*. In this species progeny survival within a second egg clutch, equal in size to the first, was approximately 42% (each clutch comprising 20 eggs), 78% (each clutch comprising 10 eggs) and 83% (each clutch comprising four) when the first and second clutches were 'laid' simultaneously (they were placed on hosts by the experimenter, see below). However, when the time between 'ovipositions' was 12 h or more, progeny survival within the second clutch was reduced to less than 10% for clutches comprising either 10 or 20 eggs (Fig. 2.62).

Experiments aimed at investigating the mortality effects of superparasitism in a gregarious species can be conducted along the lines described in the section on superparasitism in relation to growth and development rates. Sex differences in survival may be examined in such experiments; several studies (Vinson & Iwantsch, 1980b) have shown that with increased larval crowding there is a tendency for preferential survival of males.

Superparasitism in egg parasitoids can be investigated using in vitro techniques (Strand & Vinson, 1985; Strand et al., 1986; Marris & Casperd, 1996); parasitoid eggs and larvae can be added to a volume of culture medium equivalent in volume to a host egg.

As noted in Chap. 1 (Sect. 1.9), the survival chances of parasitoid immatures can in some case be *improved* by superparasitism. For example, of

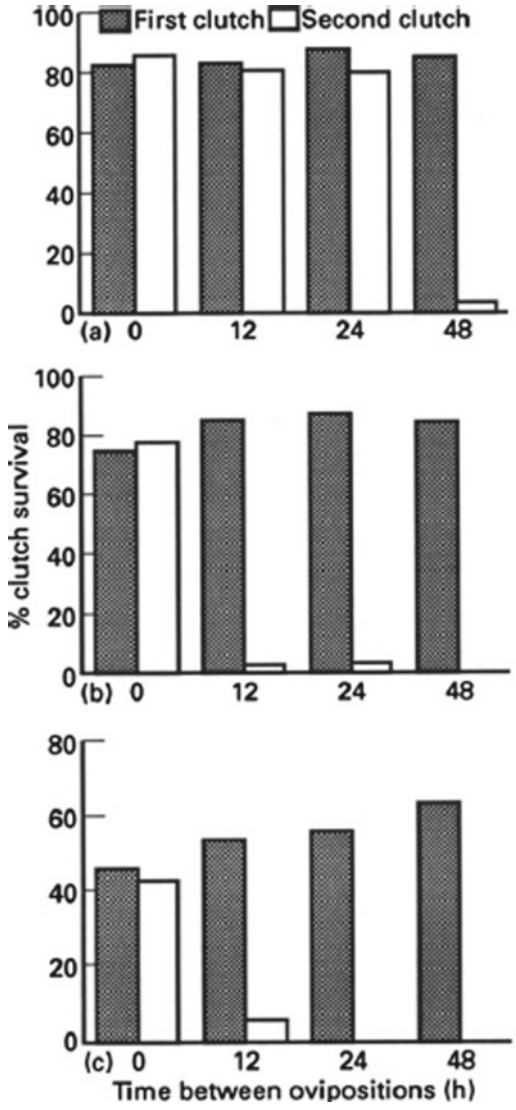


Fig. 2.62 The relationship between progeny survival within first and second clutches of eggs, and the time between 'ovipositions' for starting clutches of **a** 4; **b** 10; **c** 20 eggs, in the gregarious parasitoid wasp, *Habrobracon hebetor* (Braconidae). First and second clutches were equal in size for each experiment. *Source* Strand and Godfray (1989). Reproduced by permission of Springer Verlag

eggs of *Asobara tabida* laid in larvae of *Drosophila melanogaster*, 1% survive in singly parasitised hosts whereas 7% survive in superparasitised hosts (van Alphen & Visser, 1990), encapsulation being the principal cause of mortality in both cases. Van Strien-van Liempt (1983) measured the survival of *Asobara tabida* and *Leptopilina heterotoma* in multiparasitised *Drosophila* hosts and compared these values with survival in singly parasitised hosts. Percentage survival in instances of multiparasitism was not always lower than survival in singly parasitised hosts; in most cases, multiparasitism provided a mutual survival advantage. In cases such as these where parasitoid survival is increased through superparasitism, the mechanism is thought to be exhaustion of the host's supply of haemocytes (see Host Physiological Defence Reactions, for further discussion).

Compared with an Israeli strain, a Californian strain of the aphelinid *Comperiella bifasciata* was subject to a higher encapsulation rate in red scale and also superparasitised more hosts. Blumberg and Luck (1990) suggested that since the risk of encapsulation is reduced in superparasitised hosts (see also Sagarra et al., 2000a), the higher degree of superparasitism shown by the Californian strain is a strategy to avoid encapsulation.

For a study of intra- and interspecific larval interactions among a subweb of dipteran (specialist and generalist tachinid) and hymenopteran parasitoids, and their consequences for parasitoid survival, see Iwao and Ohsaki (1996). The mortality effects of superparasitism can be expressed as *k*-values (Sect. 7.3.4).

2.10.2.9 Host Physiological Defence Reactions

Introduction

Endoparasitoid larvae and eggs may die owing to a reaction of the host's immune system. The term immune system is used in the loose sense that the hosts are capable of mounting a defensive response against foreign bodies. The response does not involve either a specific 'memory', with accelerated rejection of the second of two sets of an introduced foreign tissue, or a marked increase in the concentration of some specific humoral component, as has been shown for vertebrates. Thus, the probability of a parasitoid

eliciting an immune response in an insect is independent of previous challenges (Boulétreau, 1986).

Host defence reactions are of several kinds (Strand & Pech, 1995; Carton & Nappi, 1997; Quicke, 1997; Fellowes & Hutcheson, 2001; Strand, 2008; Smilanich et al., 2009, for reviews), but the most commonly encountered type of reaction is encapsulation. Usually in encapsulation the foreign invader becomes surrounded by a multicellular sheath composed of the host's haemocytes (Fig. 2.63). Successive layers of cells can often be discerned, and on the outer surface of the parasitoid egg or larva there often develops a necrotic layer of melanised cells, representing the remnants of the blood cells that initiated the encapsulation reaction. The melanin deposits on the surfaces of encapsulated parasitoid eggs and larvae often provide the first clue to the occurrence of encapsulation (Fig. 2.63a–c). Parasitoid immatures die probably from asphyxiation, although starvation may be the principal cause of death in some cases. Phagocytosis of parasitoid tissues gradually occurs, at least during the initial stages of encapsulation.

Parasitoids can resist, i.e., evade and/or supress the immune responses of their hosts (Quicke, 1997; Beckage, 1998a, b, 2008; Kraaijeveld et al., 1998; Fellowes & Hutcheson, 2001; Schmidt et al., 2001; Schmidt, 2008; Strand & Burke, 2014; Ye et al., 2018). One means of evasion is the laying of eggs in refuges from encapsulation. Some parasitoids oviposit into specific host organs such as the nerve ganglia and salivary glands, where an egg cannot be reached by the host's haemocytes (Strickland, 1923; Salt, 1970; Rotheray, 1979; Dijkerman, 1988). Many early-instar parasitoid larvae, which are also potentially exposed to a host's immune defences, migrate to specific regions of the host after they hatch from the egg (the first-instar larvae of many ichneumonids use their caudal appendage for this purpose) (Salt, 1968). In other parasitoids, the immature stages have surface properties that prevent encapsulation (Rotheram, 1967; Salt, 1968). The risk of encapsulation in a particular

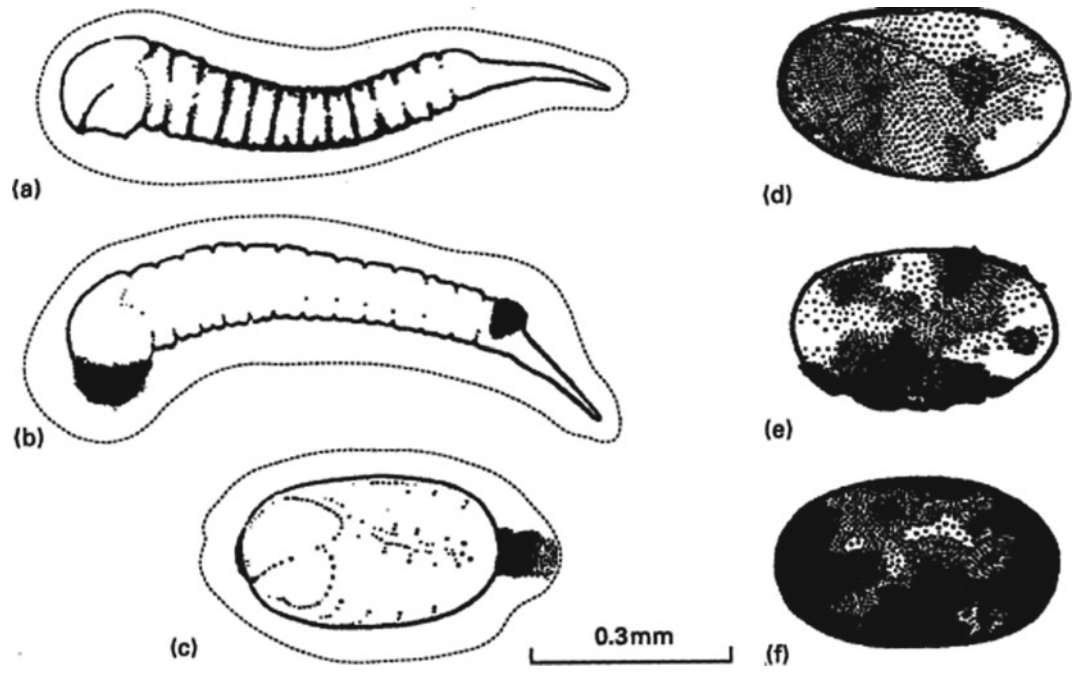


Fig. 2.63 Encapsulation and melanisation: **a**–**c** encapsulated larvae and egg of *Venturia canescens* implanted in a non-host insect (deposits of melanin can be seen); **d**–**f** deposits of melanin on eggs 24, 32 and 48 h after implantation in a non-host insect. *Source* Salt (1970). Reproduced by permission of Cambridge University Press

host species can be drastically increased by washing the surface of the parasitoid eggs using either solvents or water before they are artificially injected. Eggs of *Venturia canescens* removed from the ovarioles are encapsulated if they are artificially injected into the haemocoel of *Anagasta*, whereas eggs removed from the calyx region of the lateral oviduct do not become encapsulated. In some parasitoids the ovipositing female or her offspring are able to manipulate or disrupt the immune system (see reviews by Strand & Pech, 1995; Lavine & Beckage, 1996; Beckage, 1998a, b). Population genetic and dynamic aspects of encapsulation are discussed by Boulétreau (1986), Godfray and Hassell (1991), Kraaijeveld et al. (1998) and Fellowes and Godfray (2000); see also Chap. 3.

Encapsulation is usually studied in vivo in either laboratory-cultured or field-collected hosts. However, some workers have successfully used *in vitro* techniques (e.g., Ratner & Vinson, 1983; Benson, 1989; Lovallo et al., 2002).

Host Populations and Species

The ability of a host to encapsulate parasitoids is genetically determined (Chap. 3) and there may be considerable variability in encapsulation rate between populations of a host species (Boulétreau, 1986; Maund & Hsiao, 1991; Kraaijeveld & van Alphen, 1994, 1995a; Hufbauer, 2001; see also Dijkerman, 1990). For example, in *Drosophila melanogaster* there are clear differences between fly populations from different parts of the world with respect to the frequency with which *Leptopilina boulardi* is encapsulated (Boulétreau, 1986). Such effects are an important consideration when one is planning to release biological control agents (Maund & Hsiao, 1991).

The risk of encapsulation also varies between host species. The ability of a parasitoid species to avoid encapsulation may determine at least partly: the range of host species that it parasitises in nature, and also the different levels of successful parasitism recorded among these hosts (e.g., Heimpel et al., 2003). The relevance of this to classical biological control introductions is discussed by Alleyne and Wiedenmann (2001). Differential mortality in different host species due to encapsulation may have played an important role in the evolution of host specificity, including preferences, of many endoparasitoids. Dijkerman (1990) observed that the abundance of *Diadegma armillata*, a solitary endoparasitic ichneumonid, in the parasitoid complexes associated with *Yponomeuta* moths, varies among host species, being high in the complex associated with *Y. evonymellus* and very low in that associated with *Y. cagnagellus*. To determine whether this variation corresponds with the ability of each host species to encapsulate the parasitoid, Dijkerman (1990) used the following methods:

A. Parasitism experiments: Larvae of the different moth species were exposed to female *D. armillata*. Several days later, a sample of the hosts was taken and the insects dissected. The remaining hosts were maintained until the parasitoids emerged. By dissecting the hosts, the presence of parasitoid eggs or larvae was recorded, and the following noted:

1. The rate of infestation, i.e., the number of host larvae containing at least one egg of *D. armillata* as a percentage of the total number of larvae dissected.
2. Percentage encapsulation: [the number of encapsulated progeny divided by the total number of eggs found at dissection] $\times$ 100 (this measure of encapsulation efficiency might be less useful in cases where there is a high and variable degree of superparasitism among hosts, which was not the case in this study, see below).

By rearing hosts, the following were measured:

3. The rate of successful parasitism: [the number of host individuals yielding *D. armillata* adults divided by the total number of *Yponomeuta* yielding moths or parasitoids] $\times$ 100;
4. The percentage mortality of larvae: [the number of larvae dying during their

development divided by the initial number of parasitoid larvae] × 100 (note that if the mean number of parasitoid eggs per parasitised host significantly exceeds 1.0, a correction factor will need to be applied to the data to allow for the effects of parasitoid mortality through superparasitism).

Simultaneously, under the same conditions, host larvae that were not exposed to parasitoids were reared to moth emergence. This was done to establish whether the results could be biased by a higher mortality of parasitised hosts, compared with unparasitised hosts, in rearings.

B. Dissections of field-collected late-instar, hosts: The following were recorded:

5. Percentage encapsulation (see above); percentage of successful attacks (successful at the time of dissection, notwithstanding encapsulation later on), calculated as: [the number of parasitoid eggs or larvae recorded at dissection, divided by the total number of hosts dissected] × 100. To exclude the potentially confounding effects of time and place, comparisons were made only for samples collected at the same locality and same time of day.

Except for one species, *Y. evonymellus*, infestation rates and successful parasitism rates recorded in the laboratory were markedly different. In *Y. mahalebellus* and *Y. plumbellus* no wasps were reared despite infestation rates of 30% and 95%, whereas in *Y. evonymellus* almost all infested larvae yielded adult parasitoids. Since mortality of parasitised hosts was not different from that of control larvae, and the mean number of parasitoid eggs per parasitised host was little more than 1.0, the differences between infestation and successful parasitism could be explained in part by encapsulation. The field dissections revealed that *Y. cagnagellus* suffers fewer successful attacks than *Y. evonymellus*, despite being the more abundant species at some localities. The low successful parasitism in *Y. cagnagellus* corresponds with the very low probability of survival of *D. armillata* in that species. An interesting footnote to Dijkerman's (1990) findings is the observation that all of the *Yponomeuta* species in which there was a high rate of encapsulation of *D. armillata* are considered to have diverged early in the evolution of the genus, whereas the more recently evolved moth species show either an intermediate rate of encapsulation or do not encapsulate eggs at all (Dijkerman, 1990).

An alternative approach was taken by Benson (1989), who used an in vitro technique. He tested the eggs of three aphidophagous ichneumonid species (Diplazontinae) against the haemolymph of a range of hover-fly species. The host ranges and preferences (including behavioural preferences) of each species were already well known, and this enabled rank orders of reaction to be predicted. Haemolymph from a host species was mixed with insect tissue culture fluid and an egg of a diplazontine was added. When 24 h had elapsed, the fluid was examined for changes in colour, the extent of the change, and the formation of a capsule. The predictions for each parasitoid species in different hosts and for each host species with different parasitoids were confirmed, strongly suggesting that differential host suitability has played a significant role in determining host specificity in diplazontine ichneumonids.

Heimpel et al. (2003) make a distinction between 'suitable' hosts, in which most or all parasitoid progeny can complete development, and 'marginal' hosts, in which a substantial fraction of host individuals is able to debilitate the immature parasitoids and survive, and point out that marginal hosts may act as a 'sink' for parasitoid eggs. The ecological significance of this effect was explored through modelling by Heimpel et al. (2003). Note, however, that 'suitability' was used by Heimpel et al. (2003) in a narrow sense for the purposes of their study; 'suitability' *sensu lato* (broad sense) encompasses constraints upon larval growth and survival, as well as upon survival.

Host Plant

The rate of encapsulation of a parasitoid in a particular host species may vary with the species

of plant that the insect feeds on (Ben-Dov, 1972; Blumberg, 1991; Soussi and Le Ru, 1998, but see Blumberg et al., 1995). For example, the scale insect *Protopulvinaria pyriformis* encapsulates a larger percentage of eggs *of Metaphycus stanleyi* when grown on *Hedera helix* or *Schefflera arboricola* than when grown on avocado plants (Blumberg, 1991). Similarly, the mealybug *Pseudococcus affinis* encapsulates a higher proportion of the eggs of the encyrtid *Anagyrus pseudococci* when reared on *Aeschynanthus ellipticus* than when reared on *Streptocarpus hybridus* (Perera, 1990). Blumberg et al. (1995) did not find a host plant effect for *Anagyrus pseudococci* in their study.

Host Stage and Age

With many endoparasitoids the probability of encapsulation occurring increases with host stage or host age (Berberet, 1982; van Alphen & Vet, 1986; Slansky, 1986; Van Driesche, 1988; Dijkerman, 1990; Strand & Pech, 1995; Sagarra et al., 2000a). An explanation given by Salt (1968) for such a relationship is that earlier stages have fewer haemocytes available than later ones. Host stage does not affect the probability of encapsulation of *Habrolepis rouxi* (Encyrtidae) in its red scale hosts (Blumberg & DeBach, 1979). Note that insect eggs lack a cellular defence response to foreign bodies (Salt, 1968, 1970; Askew, 1971; Strand, 1986; Quicke, 1997).

Superparasitism

The reduction in encapsulation ability of a host with superparasitism has already been discussed. Askew (1968) drew attention to this phenomenon. Explanations given in the literature are that the host is 'weakened' or that its supply of haemocytes becomes exhausted as a result of the increased parasitoid load.

Temperature

In some parasitoid species, the temperature at which the host is reared does not affect the frequency at which encapsulation occurs (e.g., *Habrolepis rouxi*: Blumberg & DeBach, 1979; *Aprostocetus ceroplastae*: Ben-Dov, 1972; *Anagyrus pseudococci*: Blumberg et al., 1995; *Aphidius* spp.: Stacey & Fellowes, 2002), whereas in others it does (e.g., *Apoanagyrus diversicornis:* Van Driesche et al., 1986; *Metaphycus stanleyi*: Blumberg, 1991; see also Blumberg & Van Driesche, 2002). In *A. diversicornis* the rate of encapsulation is highest at the lower of two temperatures, whereas in *M. stanleyi* it is highest under high temperature regimes (Fig. 2.64). It follows that, for some species, there may be seasonal or geographical variations in encapsulation rate.

The Costs of Counterdefences to Host Resistance

While significant insights have been gained into the costs, to the host, of physiological resistance to parasitoid immatures (Fellowes & Hutcheson, 2001, provide a review), little is known about the costs of counterdefence in parasitoids. Kraaijeveld et al. (2001) sought evidence for the costs of counterdefence by *Asobara tabida* against *Drosophila* (see their paper for a protocol which involved artificially selecting populations); the only cost they could detect was the delay in hatching of the eggs (which results from them being embedded in host tissue, a defence against host haemocytes); this delay will, Kraaijeveld et al. (2001) conclude, reduce the chances of parasitoid survival if another parasitoid egg is laid in the same host. No cost was recorded in terms of either mean adult size, fat content or egg load of *A. tabida*.

2.10.3 Effects of Physical Factors on Survival of Immatures

2.10.3.1 Temperature

Parasitoids may be more hot or cold hardy than their hosts, in which case the lethal range of temperatures for the host will determine parasitoid survival. Prolonged exposure to extreme temperatures will kill the host first, and the parasitoid will then die as a result of starvation, anoxia or host putrefaction. Prior to death, a

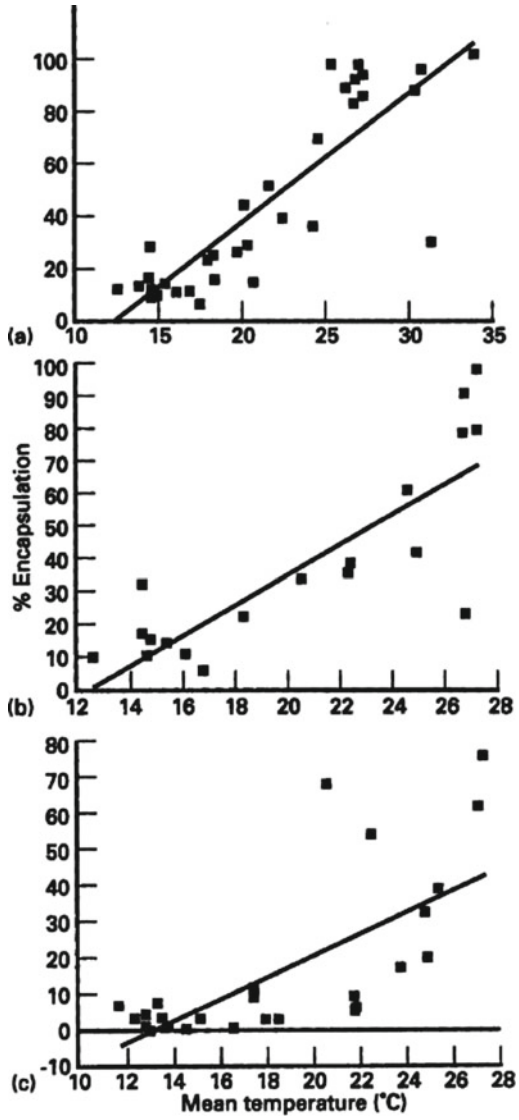


Fig. 2.64 The relationship between the rate of encapsulation of eggs and mean temperature in *Metaphycus stanleyi* (Encyrtidae) parasitising the pyriform scale on: **a** *Hedera helix*, **b** *Schefflera arboricola*, under glasshouse conditions; **c** avocado in an orchard. *Source* Blumberg (1991)

parasitoid's growth and development may be increased or decreased by the extreme temperature (Tingle & Copland, 1988).

On the other hand, parasitoids may be less hot or cold hardy than their hosts, such that they cannot tolerate the extremes of temperature that the host can tolerate, and so die as result of thermal stress. Parasitised hosts may theoretically even seek out warmer than optimal sites, raising their body temperature with the potential result that the parasitoid is killed ('behavioural fever') (Karban, 1998; see Elliot et al., 2002, and Ouedraogo et al., 2003 on behavioural fever employed by locusts to suppress fungal pathogen infection).

Within the range of temperatures that are not immediately lethal to predator larvae, the lower the temperature, the longer totally starved larvae will be able to survive, and, in the case of larvae that have prey available, the less food larvae will require to stay alive (Lawton et al., 1980). Kfir and van Hamburg (1988) have shown that the outcome of heterospecific superparasitism can be influenced by temperature. The influence of temperature on the host's ability to encapsulate parasitoids is discussed in the previous section.

2.10.3.2 Humidity

Low humidity can cause death of ectoparasitoid and predator larvae directly through desiccation (as with adults, small-bodied insects will be more prone to desiccation, all else being equal, due to their higher surface area to volume ratio), whereas high humidity can cause death indirectly by encouraging the growth of fungal pathogens.

2.10.3.3 Photoperiod

Photoperiod, because of its influence upon diurnal activity and therefore consumption rate, could affect survival of larval predators. Urbaneja et al. (2001a) found no evidence for an effect of photoperiod on survival in the parasitoid *Cirrospilus* sp. near *lyncus* (Eulophidae).

2.11 Intrinsic Rate of Natural Increase

2.11.1 Introduction

The parameter known as the 'intrinsic rate of natural increase' describes the growth potential of a population under a given set of environmental conditions. It is often used, both by ecologists (Gaston, 1988) and by biological

control workers (Messenger, 1964b), as a comparative statistic. In a biological control programme, practitioners may be faced with a choice of candidate parasitoid species; in the absence of other criteria they would select, for obvious reasons, the species with the greatest value for the intrinsic rate of natural increase (Chap. 7).

This population growth parameter is calculated, as described below, from age-specific survival and fecundity schedules. To understand first what it represents, we need to consider the most general of all population growth models, the exponential equation:

$$\frac{dN}{dt} = rN \tag{2.19}$$

where N is the number of individuals in the population at any given time t, and r is the intrinsic rate of natural increase or the instantaneous *per capita* change in population size. Under conditions of an unlimited environment and with a stable age distribution, r is a constant.

For a given species, r can take a number of values. In theory at least, the species has an optimal natural environment in which its r will attain the maximum possible value, r_m, with a stable age distribution.

2.11.2 Calculating r_m for a Parasitoid Wasp Species

R_m is calculated by iteratively solving the following equation:

$$\sum_{x=0}^{n} e^{-r_m x} l_x m_x = 1 \tag{2.20}$$

where x is the mid-point of age intervals in days, l_x is the fraction of the females surviving to the pivotal age x (or, put another way, the probability of a female surviving to age x), m_x is the mean number of female 'births' during age interval x per female aged x, and e is the base of natural logarithms. Trial r_m values are substituted into the above expression until the left-hand side is (arbitrarily) close to 1.

l_x and m_x are calculated by tabulating (Table 2.2) age-specific fecundity and age-specific survival data obtained from cohort fecundity and survival experiments (Sects. 2.7.2 and 2.8.1 discuss the experiments; a graphical display of such data is given in Fig. 2.65). If we find from examination of the life-table that only 50% of wasps survive to the age of 5 days, then $l_5 = 0.5$. If we find that the average number of female offspring produced per individual alive during the age interval x is 25, then $m_{25} = 25$ (see caption to Table 2.2, for calculations based on another data set). The mean time taken from oviposition to adult eclosion, which can be measured in a separate experiment, is added to the pivotal age of each female. For example, this time period was 12.5 days for *Aphidius smithi* at 20.5 °C (Mackauer, 1983). Parasitoid mortality during the immature stages also needs to be measured. In *A. smithi* this mortality was negligible, so the probability of being alive at pivotal age 12.5 days + 1 day was set equal to 1.0 for all females (Mackauer, 1983). In *Aphidius sonchi* the time from oviposition to adult eclosion was 11.3 days and mortality of immatures was 8.0%, so the probability of being alive at pivotal age 11.3 days + 1 day was set equal to 0.92 (Liu, 1985b).

Once the values for l_x and m_x are calculated, then the following population statistics can also be calculated (Messenger, 1964b):

1. The gross reproductive rate, GRR = $\sum m_x$ (the mean total number of eggs produced by females over their lifetimes, measured in females/female/generation);
2. The net reproductive rate, or 'basic reproductive rate' (the number of times a population will multiply per generation) $R_o = \sum l_x m_x$ (measured in females/female/generation);
3. The finite capacity for increase, $\lambda = e^{r_m}$ (the number of times the population will multiply itself per unit of time; measured in females/female/day);
4. The mean generation time, ($T = (\log_e R_o)/r_m$ (measured in days);

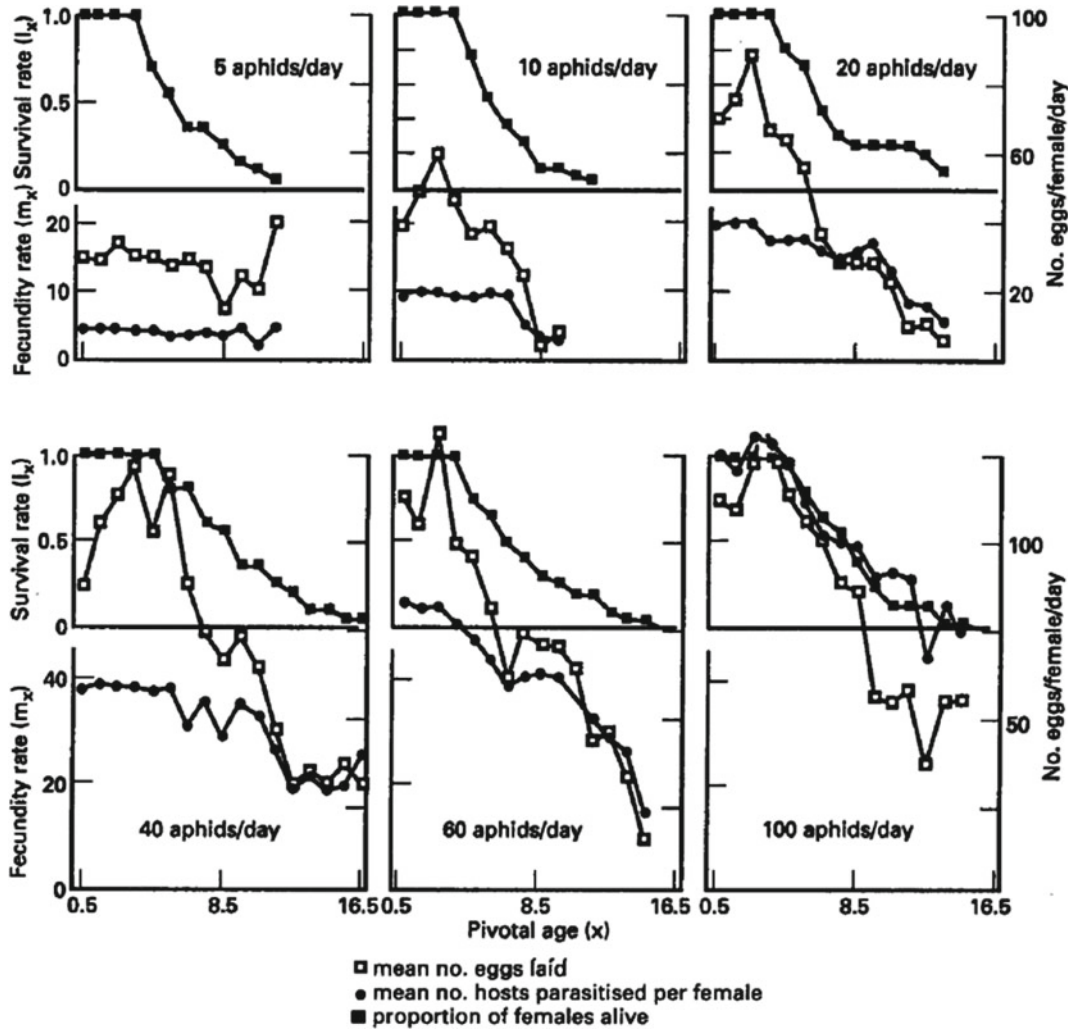


Fig. 2.65 Age-specific fecundity and survival rates of *Aphidius smithi* provided with different densities of its host *Acyrthosiphon pisum*. *Source* Mackauer (1983). Reproduced by permission of The Entomological Society of Canada

5. The doubling time (DT = $\log_e 2/r_m$ (the time, measured in days, required for a given population to double its numbers).

Using the data in Table 2.2, r_c = 0.289, r_m = 0.296, GRR = 108, R_o = 71.2, λ = 1.344, T_c = 14.74 (see below for explanation of r_c and T_c), T = 14.41, DT = 2.24. Statistical and computational aspects of the estimation of r_m are discussed by Maia et al. (2000). These authors also provide an SAS program that uses the jackknife technique.

r_m can be measured (in female/female/day) for each of a range of host densities. It increases with increasing host density (Mackauer, 1983; Liu, 1985b). In *Aphidius smithi* this increase is also reflected in λ and also DT, which was less than half as long at the highest than at the lowest host density (Mackauer, 1983). Because in both *A. smithi and A. sonchi* the ovipositional pattern and the pattern of survival were similar to one another at the different densities (Fig. 2.65), host density showed no significant effect on T.

Table 2.2 Hypothetical life-table for an experimental cohort of female parasitoids. x is the mid-point of age intervals (pivotal age) in days, l_x is the fraction of the females surviving to age x (in this example we assume no deaths occurred during development, so the proportion of females surviving to commence ovipositing is 1.0), and m_x is the mean number of female 'births' during age interval x per female aged x

x	l_x	m_x	$l_x m_x$
12.5	1.0	12	12.0
13.5	0.9	14	12.6
14.5	0.8	18	14.4
15.5	0.7	22	15.4
16.5	0.5	25	12.5
17.5	0.3	13	3.9
18.5	0.1	4	0.4
			$\sum l_x m_x = R_o = 71.2$

To obtain a true measure of the influence of host density on the parasitoid's population statistics, some authors have based the m_x values on the number of hosts *actually* parasitised ('effective eggs' of Messenger, 1964b). This takes account of superparasitism; thus the number of hosts parasitised can be assumed to equal the number of progeny eventually produced (ignoring cases where no parasitoid progeny succeeds in developing in a parasitised host).

Another factor that needs to be taken into account is the sex ratio of the progeny. This can be achieved by multiplying all m_x values in the life-table by the overall population sex ratio, P, which is the proportion of females in all offspring produced. Regression of r_m on the natural logarithm of host density for different values of the sex ratio gives a series of parallel lines (Mackauer, 1983; Liu, 1985b; Tripathi & Singh, 1991) (Fig. 2.66 shows regressions obtained for *Aphidius smithi*). The variation in r_m as a function both of the parasitoid's sex ratio and of host density can be shown as a response surface (Fig. 2.67 shows the response surface for *Aphidius sonchi*) (Mackauer, 1983, gives details of the statistical procedure involved in obtaining the response surface). As can be seen from Fig. 2.67, r_m increases as either host density or sex ratio increases, and at a given value of P the rate of increase in r_m slows at higher host densities. In *A. sonchi* the deceleration in r_m at high densities is such that the percentage increase in host density required to obtain a given percentage increase in r_m is constant. For example, at $P = 0.70$, a 20% increase in r_m from 0.25 to 0.30 requires an increase in host density from 15 to 39 per day, i.e., 24 hosts, while a 20% increase in r_m from 0.30 to 0.36 requires an increase in host density from 39 to 101 per day, i.e., 62 hosts. This rule applies over the whole range of $0 \leq P \leq 1.0$, although the required increment in host density increases in absolute terms as the value of P declines. When $P = 0.40$, an increase of 48 hosts, from 30 to 78 per day, is required to obtain a 20% increase in r_m from 0.25 to 0.30.

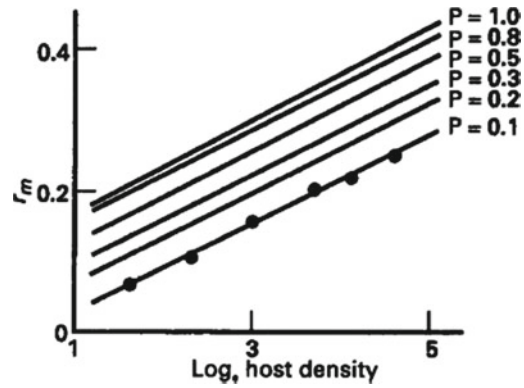


Fig. 2.66 The relationship between the intrinsic rate of natural increase (r_m) *of Aphidius smithi* (Braconidae) and natural logarithm of host density, for different overall sex ratios. *Source* Mackauer (1983). Reproduced by permission of The Entomological Society of Canada

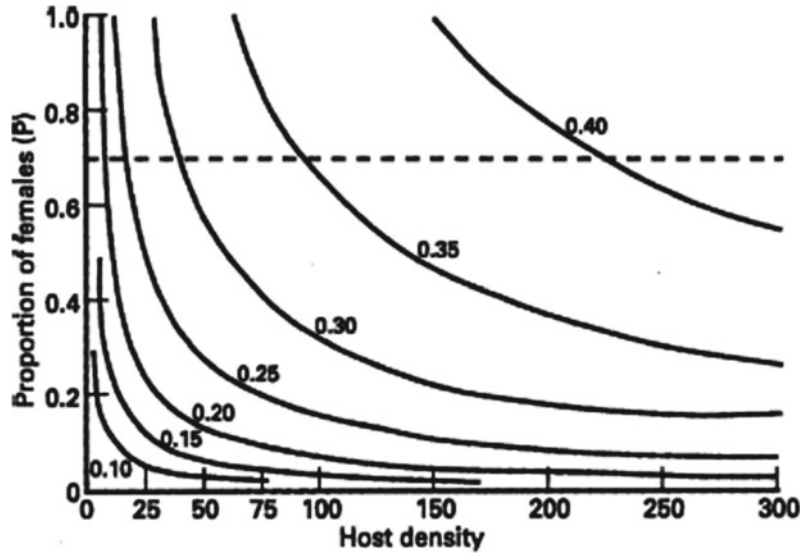


Fig. 2.67 Response surface showing lines of equal r_m for *Aphidius sonchi* (Braconidae) for different host densities and parasitoid sex ratios (P, proportion of females). The broken line indicates a sex ratio of $P = 0.7$ observed in the laboratory. *Source* Liu (1985b)

The r_m of *Hyperomyzus lactucae*, the host of *A. sonchi*, is 0.3375. For a P value of 0.7, which is the sex ratio for *A. sonchi* in laboratory cultures, the parasitoid will achieve an r_m of 0.3378 at a host density of 74/day (preferably, the field sex ratio should be used in this computation, Mackauer, 1983). If the host density is increased to 200 per day, a sex ratio as low as 0.3 will yield an r_m value of 0.3367, which is again close to that of the host.

Assuming an absence of superparasitism (which is typically higher at low densities), the parasitoid's realised m_x will be equal to its oviposition rate, so yielding values of r_m higher than those computed. The minimum host density required to eliminate egg wastage through superparasitism can be determined. Theoretically, at that density the parasitoid's r_m will reach a maximum value that can be computed by setting m_x equal to the daily totals of eggs laid at the highest oviposition rate (Mackauer, 1983, gives details of the statistical procedure involved).

Knowing how r_m varies in relation to factors such as host density (see above) and temperature (see below) can help biological control practitioners in deciding on the timing of introduction, for example in an inoculative release programme.

Equation 2.20 is not very 'transparent', that is, it is not particularly useful for any broad consideration of the relation between r_m and 'synoptic' life-history parameters such as generation times (Laughlin, 1965; May, 1976). A more useful statistic is r_c, the capacity for increase, which is an approximation for r_m. It is calculated as follows:

$$r_c = \frac{\log_e R_o}{T_c} \tag{2.21}$$

where T_c is the cohort generation time, defined as the mean age of maternal parents in the cohort at birth of female offspring (Laughlin, 1965; May, 1976) (for a discussion of the relationship between T and T_c, see May, 1976):

$$T_c = \sum_x l_x m_x / R_o \tag{2.22}$$

Equation 2.21 is based on the assumption that the reproductive period is brief relative to the total life-cycle, which results in a small error in the estimation of generation time. r_c is a good approximation for r_m when R_o and thus population size remains approximately constant, or when there is little variation in generation length, or for some combination of these two factors (May, 1976).

A relatively simple method for calculating values for r_c was developed by Livdahl and

Sugihara (1984). It dispenses with the need to construct detailed survivorship and fecundity schedules, and uses indirect estimates of R_o and T_c. It assumes the organisms being studied to have a Type III survivorship curve for the whole life-cycle, with high larval mortality and negligible adult mortality through the reproductive period; this assumption is only partly satisfied in the case of parasitoids, since in the laboratory there is likely to be low larval mortality while in the field there is likely, in many species, to be high mortality of females during the reproductive period. To use Livdahl and Sugihara's (1984) method, one only needs to observe cohorts during the maturation period in order to obtain measurements of the number of newly emerged adult females and their average size.

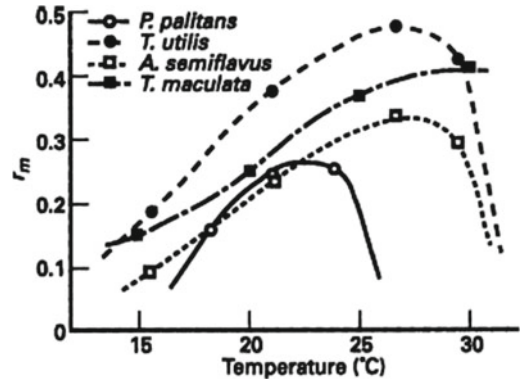


Fig. 2.68 Comparison of intrinsic rate of natural increase (r_m) of the aphid parasitoids *Praon palitans*, *Trioxys utilis* (Braconidae), *Aphelinus semiflavus* (Aphelinidae) and their aphid host, *Therioaphis maculata*, over a range of constant temperatures. *Source* Force and Messenger (1964). Reproduced by permission of The Ecological Society of America

2.11.3 Effects of Host or Prey Species and Stage

Host stage and species, through their effects on body size in parasitoids, influence life-history variables such as fecundity and longevity, so they would be expected to affect r_m and r_c. This is indeed the case: see Cloutier et al. (2000) on r_m in *Aphidius nigripes*, and Yu et al. (1990) on r_c in *Encarsia perniciosi*. In both species, the intrinsic rate of natural increase/capacity for increase was higher in larger hosts.

Prey species can also be expected to influence the intrinsic rate of natural increase of predators, as has been confirmed, for example, by Venzon et al. (2002) for the bug *Orius laevigatus* and Fathi (2009) for *Orius niger* and *O. minutus*.

2.11.4 Effects of Temperature

Since larval development rate, female survival and female fecundity vary with temperature (Sects. 2.7.3, 2.9.3 and 2.8.4) we would expect r_m to vary also, which is the case. Figure 2.68 shows how r_m varies with temperature in three species of parasitoid and their aphid host (Force & Messenger, 1964).

For examples of other studies, see Geusen-Pfister (1987) (*Episyrphus balteatus*), Cave and Gaylor (1989) (*Telenomus reynoldsi*), Lohr et al. (1989) (*Apoanagyrus lopezi*), Smith and Rutz (1987) (*Urolepis rufipes*), Mendel et al. (1987) (*Anastatus semiflavidis*), Miura (1990) (*Gonatocerus cinticipitis*), Cocuzza et al. (1997b) (*Orius* spp.), Urbaneja et al. (2001b) (*Cirrospilus* sp.), Ren et al. (2002) (*Nephaspis oculatus*), Seal et al. (2002) (*Catolaccus hunteri*), Roy et al. (2003) (*Stethorus punctillum*), Pakyari et al. (2011) (*Scolothrips longicornis*) and Souna et al. (2021) (*Therophilus javanus*).

Siddiqui et al. (1973) provide a model to describe the relationship of $1/r_m$ to temperature. Using data for *Aphidius matricariae*, one of us (M.J.W. Copland) found the model to provide a good fit to the data over part of the temperature range only. A simple polynomial model could express the relationship much more accurately.

2.12 Dormancy

2.12.1 Introduction

Life-cycles in most insects are characterised by profound season-related changes in growth,

developmental and reproductive characteristics. Different species possess unique sets of eco-physiological responses that regulate seasonal cycles, facilitating temporal synchrony with seasonal variations in the availability and state of biotic and abiotic factors in their habitat (Tauber et al., 1986, 1994). Understanding seasonal changes in the growth, development and reproduction of insect natural enemies is also an important tool in applied ecology. In particular, it is necessary to investigate the degree of synchrony between generations of pests and beneficial insects in order to determine the best strategies for successfully mass-rearing, storing and releasing biological control agents (van Lenteren, 1986; Chang et al., 1996; Ringel et al., 1998; Chap. 7). This is particularly true for parasitoids, most of which have very limited host ranges (Askew, 1971; Godfray, 1994; Quicke, 1997) and are therefore closely synchronised with successive generations of their hosts.

Particularly in temperate environments, insects enter a dormant state during unfavourable periods e.g., winter. Dormancy in insects occurs in a number of ways that differ both physiologically and ecologically. Types of dormancy are generally classified according to whether they are obligate and/or seasonally recurring (diapause and aestivation) or facultative in nature, occurring in direct response to certain stimuli (quiescence). Dormancy has been frequently reported among predators and parasitoids, where examples occur in all stages of development. Investigations of dormancy involving parasitoids are potentially much more complicated than in predatory insects, because host–parasitoid interactions occur over three, rather than two trophic levels. Furthermore, parasitoids are generally much more specific in their choice of hosts than predators are with their prey (Chaps. 1 and 6). Factors which stimulate the onset of diapause, aestivation or quiescence in parasitoids may be perceived directly by the natural enemy or indirectly in response to physiological cues released by the host. The dynamic effects and evolution of diapause in coupled parasitoid–host systems have been explored by Ringel et al. (1998) using theoretical modelling.

2.12.1.1 'Obligate' or 'Predictive' Dormancy: Diapause and Aestivation

Predictive dormancy is initiated in advance of adverse conditions, and most commonly occurs in response to predictable changes in seasonal environments (Müller, 1970). Two types of predictive dormancy have been described: diapause (during winter) and aestivation, or summer diapause (during summer). Tauber et al. (1986) define diapause as a neurohormonally mediated dynamic state of low metabolic activity associated with reduced morphogenesis, increased resistance to environmental extremes, and altered or reduced behavioural activity. Diapause occurs during a genetically determined state of metamorphosis and generally in response to token environmental cues that precede the unfavourable condition.

The important point to bear in mind is that diapause-inducing stimuli are 'registered' commonly before the diapausing stage is reached. Diapause occurs in response to changes in, and interactions between, various biotic and abiotic factors, including photoperiod, temperature, humidity, and prey or host availability. Diapause termination also requires specific environmental conditions (Tauber et al., 1993).

2.12.1.2 'Facultative' or 'Consequential' Dormancy: Quiescence

Quiescence is a reversible state of suppressed metabolic activity that occurs in response to environmental stimuli but does not involve preparatory hormonal or physiological changes in anticipation of environmental conditions. In many cases, timing and duration of quiescence are not fixed seasonally, but are highly variable and may last for many months or even years. The breaking of quiescence may require some kind of stimulation, signifying that the environment is favorable for development or activity.

Identifying, for a particular natural enemy, the nature of its dormancy, and establishing which biotic and physical factors play a role in its initiation, maintenance and termination, determining how these factors interact, and establishing

which of the insect's life-stages are sensitive to predictive dormancy-inducing factors, can be very difficult, involving in some cases complex multifactorial experimental designs. Often, knowledge of the dormancy characteristics of related species can be helpful in simplifying experiments; for example, it can help in narrowing down the list of candidate abiotic factors. We do not provide detailed advice on protocols here (for such information see Leather et al., 1993); instead, we provide a brief overview of diapause-inducing factors, supplemented with a few snippets of practical information.

2.12.2 Effects of Biotic Factors on Dormancy

2.12.2.1 Prey Availability and Quality (Predators)

Seasonal variation in the availability of prey has been reported to have a marked influence on the incidence of dormancy in predators. Aestivation in *Coccinella septempunctata* is stimulated by availability of suitable prey, as well as by other factors (Kawauchi, 1985; Zaslavsky & Vagina, 1996). Polymorphic seasonal cycles in the lacewing, *Chrysoperla carnea*, are similarly influenced by the abundance of prey (Tauber et al., 1986). In some predator species diapause incidence appears to depend on the type or the quality of the diet fed upon (Horton et al., 1998). Since prey availability is, in many predators, likely to be linked to various biotic factors, it is important to try and devise an experimental design that enables the effects of the various factors to be disentangled (and interaction effects tested for), although this may be difficult or even impossible in many cases.

2.12.2.2 Host Physiology (Parasitoids)

Many parasitoids oviposit in nutritionally suboptimal early host stages, and their larvae exhibit developmental arrest, completing their development only after the host has moulted to the penultimate or even final instar (Vinson & Iwantsch, 1980a; Harvey et al., 1994, 1999). Developmental delays are adaptive in a number of respects. First, they reduce the selection pressure for a fixed maternal response at oviposition by allowing female parasitoids to attack a wide range of host stages rather than a single one (Cloutier et al., 1991). Second, they ensure that the host reaches a critical size and physiological condition in which the parasitoid can complete its development (Hemerik & Harvey, 1999). Finally, they synchronise parasitoid and host generations intra- and interseasonally. There remains debate as to whether developmental delays are a form of diapause or of quiescence (Lees, 1955; Mellini, 1972; Godfray, 1994). Tauber et al. (1983) argue that host-mediated developmental arrest in the first-instar parasitoid larva is a form of obligate diapause because it shares many characteristics associated with the diapause syndrome (see their paper, and also Doutt et al., 1976).

Parasitoid larvae may also enter diapause in response to dormancy-related physiological changes in the host. For example, Polgar et al. (1991) and Christiansen-Weniger and Hardie (1997, 1999) examined factors influencing diapause induction in several braconid endoparasitoids attacking different morphotypes of their common aphid hosts (see also Polgar & Hardie, 2000). Parasitoids tended to enter diapause more in sexual hosts (oviparae) which occur in late summer, than in asexual hosts (virginoparae) which occur earlier in the season. Diapause appeared to be initiated mostly by hormonal differences between different aphid morphs. Diapause in idiobiont parasitoids has been reported to be influenced by the diapause status of their host in some associations (McNeil & Rabb, 1973; Strand, 1986), but not in others (Mackay & Kring, 1998).

The incidence of diapause among parasitoid progeny can vary with host species (Kraaijeveld & van Alphen, 1995b).

2.12.3 Effect of Physical Factors on Dormancy

2.12.3.1 Photoperiod

Insect natural enemies, like other insects, are very sensitive to the duration and intensity of light exposure. In temperate regions, photoperiod is a major factor controlling diapause initiation, maintenance and termination in insects (Tauber et al., 1983, 1986). Danilevskii (1965) defined the 'critical photoperiod' as that which elicits a >50% response amongst individuals in a population.

Many heteropteran bugs overwinter in a state of reproductive diapause as adults, and typically diapause is induced by the photoperiod during nymphal development, although the adult stage may also be sensitive (Yeargan & Barney, 1996; Ruberson et al., 2000). The multivoltine coccinnellid *Coccinella septempunctata*, which is widely distributed over much of the Palearctic, undergoes aestivation as first-generation adults, from April to August, in response to increasing day length (Sakurai et al., 1986; Katsoyannos et al., 1997) (this is immediately followed by a variable period of quiescence during winter). Photoperiod is also reported to be an important diapause-inducing stimulus for odonates (Norling, 1971; Pritchard, 1989).

In parasitoids, many studies have reported a key role for photoperiodic induction of dormancy (reviewed by Askew, 1971; Tauber et al., 1983; Godfray, 1994; Quicke, 1997). Field sampling of hosts is a useful starting point for gathering tentative evidence of the role of photoperiod in diapause induction in bivoltine endoparasitoids (Jervis, 1980).

2.12.3.2 Temperature

Temperature is another important diapause-inducing stimulus for predators. In coccinellids, diapause may be stimulated by seasonal exposure to low temperatures (Kawauchi, 1985) or be due to an interaction of temperature and photoperiod (Ongagna & Iperti, 1994). The coccinellid *Rhyzobius forestieri* does not enter diapause, but the application of a cold shock at 8 °C induces quiescence which can persist for several months if this condition is maintained (Katsoyannos, 1984).

In parasitoids, most studies have shown that temperature interacts with photoperiod in stimulating diapause induction (Brodeur & McNeil, 1989; Pivnick, 1993; Polgar et al., 1995), although some parasitoids may enter diapause in response to temperature alone (Wang & Laing, 1989). Temperature is also an important determinant in the breaking of diapause: for some species it may need to be low (amateur entomologists are well acquainted with the technique of 'chilling' insect pupae, in a refrigerator, for several weeks during the winter, before exposing them to warm indoor temperatures, to achieve a pre- or early-spring emergence of adults), whereas in others it may need to be high (e.g., Hodek & Hodková, 1988; van den Meiracker, 1994; Ishii et al., 2000).

To create more natural conditions in dormancy experiments, insects can be reared under gradually increasing temperatures (to stimulate the onset of summer aestivation) or gradually decreasing temperatures (to stimulate the onset of winter diapause).

The threshold temperature and the thermal constant (Sect. 2.9.3) for postdiapause development can be estimated for a parasitoid or predator (e.g., Trimble et al., 1990).

2.12.3.3 Moisture

Among the physical factors influencing dormancy in insects, the effects of moisture and/or humidity are the most poorly understood and least studied. This is principally because the vast majority of phenological studies have been performed in the temperate zones, where photoperiod and temperature are considered, a priori, to play major roles. Evidence is accruing that moisture plays a vital role in the maintenance of dormancy in a range of predatory insects. For example, soil moisture, acting independently or in combination with photoperiod and temperature has been shown to influence rates of development or activity (Jayanth & Bali, 1993; Bell, 1994; Bethke & Redak, 1996; Sanon et al., 1998; Nahrung & Merritt, 1999; see also review by Tauber et al., 1998).

2.12.4 The Fitness Costs of Dormancy

This is a little explored area of insect natural enemy biology. Chang et al. (1996) revealed that post-diapause adults of the lacewing, *Chrysoperla carnea* experienced higher reproductive success than individuals which had overwintered in a state of quiescence. Moreover, first-generation offspring of parents that had overwintered in diapause developed more rapidly and survived better than individuals whose parents had experienced quiescence. Ellers and van Alphen (2002) showed that in *Asobara tabida* an increase in diapause length led to higher mortality among diapausing pupae, together with decreases in egg load, fat reserves and dry weight of emerging adult females. See also Anderson (1962), on *Anthocoris nemorum*, and Leather et al. (1993) for a discussion of the costs of overwintering among insects generally.

2.13 Investigating Physiological Resource Allocation and Dynamics

2.13.1 Introduction

This section is concerned with techniques used to study both: (1) the optimal strategy for the allocation, within the adult stage of parasitoids or predators, of carried-over physiological resources, i.e., those derived from the immature phase of the life-cycle; and (2) quantitative changes in these resources during adult life, in relation to variation in environmental factors such as food availability and quality, and host/prey abundance.

2.13.2 Patterns in Resource Allocation

Intra- and interspecific differences in the pattern of resource allocation are of considerable interest, as they help ecologists and evolutionary biologists to understand why individuals and species differ in terms of key life-history traits. Negative correlations between the amounts of resources serving different life-history functions such as egg production and survival are particularly intriguing, as they imply the existence of trade-offs, and as such are evidence that life-histories are compromises. An associated goal of ecologists is to understand the integration of suites of life-history traits, and as is becoming apparent from the literature, studying patterns of resource allocation is the way forward in this quest.

Testable hypotheses relating to resource allocation include the following:

1. All else being equal, species whose females are longer-lived and which have higher resource intake prospects should invest more in building a 'sturdy body' or 'soma' (musculature and exoskeleton) at the expense of 'abdominal reserves' (principally reproductive organs and their contents i.e., eggs, together with fat body) (Boggs, 1981). Empirical support for Boggs' hypothesis comes from her study of three species of heliconiine butterflies (Boggs, 1981; see also Karlsson & Wickman, 1989; Wickman & Karlsson, 1989).
2. Among abdominal 'reserves' there will be a trade-off between those resources allocated to initial egg production and those allocated to survival (fat body and other reserves). This is predicted by general life-history, on the basis of between-function competition for limited resources (Bell & Koufopanou, 1986; van Noordwijk & de Jong, 1986; Smith, 1991; Segoli & Wajnberg, 2020). Empirical support for this hypothesis comes from the known differential allocation of carried-over larval resources to fat body storage and initial egg load in the parasitoid wasp *Asobara tabida* (Ellers & van Alphen, 1997).
3. As body size increases in parasitoid wasps, the total amount of 'abdominal reserves' increases, and allocation to both initial eggs

(initial egg load) and stored reserves increases, but the increase in allocation to initial eggs is proportionately smaller than the increase in allocation to initial reserves. For an explanation of the adaptive significance of these relationships, and how they relate to ovigeny index, see Ellers and Jervis (2003).

4. Smaller parasitoid wasp individuals suffer disproportionately, in terms of survival, the costs of not feeding, because they emerge with smaller initial reserves. This is supported by Rivero and West's (2002) study of *Nasonia vitripennis* (Sect. 2.8.3).
5. Solitary parasitoid species should allocate relatively more resources to survival (as fat reserves) than gregarious species (Pexton & Mayhew, 2002; the hypothesis is based on optimal allocation theory, e.g., Roff, 2002). This was supported by Pexton and Mayhew's study of two *Aphaereta* species.
6. Mothers should reduce egg provisioning with age (Begon & Parker, 1986; Roff, 2002). This is supported by Giron and Casas's (2003b) study of *Eupelmus vuilletti*.

2.13.3 Resource Dynamics

Quantitative changes in resources will occur during adult life, in relation to environmental factors such as extrinsic nutrient availability and quality, and host or prey availability. Behavioural ecologists in particular are interested in these changes because they know foraging decisions to be physiologically state dependent (Chap. 1), and they appreciate that foraging, mating behaviour and other activities (including dispersal) are constrained by nutrient (intrinsic and extrinsic) supply. Hypotheses relating to resource dynamics in insect natural enemies have been tested by Ellers et al. (1998, 2001), Olson et al. (2000), Rivero and West (2002), Ellers and van Alphen (2002), Giron and Casas (2003a) and Casas et al. (2003) (parasitoids), and Otronen (1995) (the predatory fly, *Scathophaga stercoraria*) (see also Legaspi et al., 1996, on the predatory bug *Podisus maculiventris*).

2.13.4 The Techniques

Measuring Allocation, Among the Total Carried-over Resources, to 'Soma' and 'Abdominal Reserves'

This can be done by measuring the dry weight, the total nitrogen content, and the total carbon content of: (1) the head + thorax + legs + wings (collectively 'soma', sensu Boggs, 1981); and (2) the abdomen ('abdominal reserves' resource pool, sensu Boggs, 1981).

The total amount of nitrogen in each body region can be measured using Kjeldahl digestion and subsequent Nesslerization (Minari & Zilversmit, 1963), while the total amount of carbon can be measured using bomb calorimetry. Better still is elemental analysis using a CHN analyser.

Measuring Allocation, Among 'Abdominal Reserves', to Initial Egg Production and Survival, and Studying Resource Dynamics

Measuring resource allocation to initial egg production, and also the subsequent qualitative and quantitative changes that occur in reproductive tissues, can be determined using modifications of well-proven techniques (van Handel, 1984, 1985a, b; van Handel & Day, 1988; Olson et al., 2000). Except in the case of small-bodied species (in which case separate individuals would have to be used), ovary protein content can be determined for one ovary, and both lipid and glycogen content determined for the other ovary. The Bradford dye-binding colorimetric micro-assay (Bradford, 1976) can be used for protein measurement, and lipid and glycogen measurement can be done using modifications of colorimetric techniques (vanillin reaction and chemical precipitation followed by hot anthrone reaction, van Handel, 1985a, b; van Handel & Day, 1988).

Measuring allocation to energy reserves, and also measuring alterations in the amounts of these resources, would involve measuring the quantities of lipid, glycogen, and stored sugars in the ovary-less abdomen (this would include haemolymph). Lipid and glycogen content can be measured as for the ovaries (see above); an

alternative method of lipid measurement is ether extraction (Ellers, 1996; Ellers & van Alphen, 1997, 2002; Eijs et al., 1998). Stored sugar content can be measured using the hot anthrone reaction (Olson et al., 2000; Fadamiro & Heimpel, 2001).

The strategy of allocation from among the pool of 'abdominal reserves' could be influenced by: (1) nutrient intake prospects (Chap. 8); (2) egg resorption capability (Sect. 2.3.4); (3) thoracic musculature resorption capability (Kaitala, 1988, and Kaitala & Huldén, 1990, for an example of flight muscle resorption in waterstriders, and see Kobayashi & Ishikawa, 1993, for histological methodology); or (d) combinations of these (Jervis & Kidd, 1986). Unless it is already one of the variables under consideration, body mass will need to be included as a covariable in data analyses. Phylogeny-based statistical methods (Sect. 1.2.3) should be employed in the case of interspecific comparisons.

If one is interested in knowing the total level of energy reserves within an insect, these can be calculated by adding the energy content of carbohydrate to that of lipids, assuming 16.74 J per milligram of carbohydrate and 37.65 J per milligram of lipid (Casas et al., 2003).

By studying carbohydrate and lipid dynamics in both field and laboratory experimental populations (freshly emerged, starved to death, fed ad libitum, partially starved), Casas et al. (2003) were able to show that *Venturia canescens* females are able to maintain a nearly constant level of energy over an extended foraging period, that they take sugars in the field, and also that lipid reserves may be limiting as lipogenesis does not occur in adults even under conditions of high sugar availability (all parasitoid wasps studied so far are unable to synthesise lipids from sugars in significant quantities, see Giron & Casas, 2003a).

2.14 Tracking Resources

Radiotracer studies, which have been applied to other insects (e.g., Boggs, 1997b, on Lepidoptera), are now being used to study the utilisation of extrinsic nutrients by parasitoid wasps (Rivero & Casas, 1999; Giron et al., 2002; Giron & Casas, 2003a). Rivero and Casas (1999) fed females of *Dinarmus basalis* on an artificial diet comprising a sugar + radiolabelled (^{3}H) amino acid solution. The liquid food was supplied in a capillary tube, and the weight of females was compared before and after feeding, so allowing the amount of radioactivity both in the insects themselves and in the eggs they laid to be related to the amount of food ingested. It was found that the maximum incorporation, into eggs, of labelled nutrients obtained via a discrete feeding event occurred with a short period of time. However, it was also found that a large proportion of the nutrient input is stored and used gradually throughout the life of the parasitoid.

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Genetics

3

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3.1 Introduction

Genetic research on insect natural enemies was, until relatively recently, rare and was essentially limited to parasitoid wasps and some coccinellid beetles. The advent of genomic and transcriptomic sequencing technologies has revolutionised the ability of researchers to investigate the underlying genetics of a variety of developmental, life-history, predatory and defensive processes in other well-known groups such as parasitoid flies, robber flies, scorpion flies and mantids. The increase in availability, and reduction in cost, of molecular biology, genetic sequencing and associated analytical tools (Sect. 3.2) is beginning to generate resources and facilitate studies. The number of studies has expanded considerably over the last decade and it is beyond the scope of this chapter to review and describe them all. Gene location, identification, sequence, variant typing, changes in expression and associated gene networks are beginning to be characterised across many insect natural enemies. Examples include insights into phylogenetic relationships within the Mecoptera (scorpionflies and hangingflies) (Li et al., 2019) ; investigation of venom evolution (reviewed by Walker et al., 2018) in the robber fly *Dasypogon diadema* (Drukewitz et al., 2019) ; studies of gut microbiomes and the impact on host health in preying mantids (Tinker & Ottesen, 2018).

In many organisms there is geographic variation in the expression of biological traits. In parasitic Hymenoptera such variation has been reported for sex ratio, host preference, oviposition strategies, superparasitism, diapause induction, virulence, developmental time, and other traits, yet despite this broad suggestive evidence for the existence of genetic variation for many traits, relatively little was known about the underlying genetics. Among the well-studied parasitic Hymenoptera, where genetics was largely neglected until relatively recently and despite, for example, sex allocation strategies (Sect. 1.11) having been intensively studied in this group, hardly anything was known about the

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underlying genetic mechanism of sex determination. That is now changing rapidly. Heimpel and de Boer (2008) review progress in understanding the genetic and cytoplasmic factors that contribute to sex determination. Zhou et al. (2021) use comparative transcriptome analysis to investigate sex-determining gene and sex-based differences in development in the parasitic wasp *Cotesia vestalis*.

3.1.1 Basic Genetics, Reproduction and Genetic Terminology

The genetic constitution of an organism is called its genotype. This includes all the genetic material in a cell, which is organised as a collection of genes on separate chromosomes that together make up the genome. In eukaryotes, the genome also includes mitochondrial DNA, found within mitochondria, the 'powerhouses' of the cell. The position of a gene on a chromosome is known as a locus (plural = loci). An organism's phenotype is the physical manifestation of its genetic traits that result from a specific genotype and its interaction with the environment. Organisms may have one copy of each of their chromosomes in each cell, known as haploid (n), two copies diploid (2n), or more than two copies polyploid (e.g., 4n, 6n and so on). Haplodiploidy, in which males are haploid and females are diploid, occurs in all ants, bees, wasps and sawflies (Hymenoptera) and thrips (Thysanoptera), as well as in a few other insect species (Sect. 3.3). In other organisms, sex may be determined by the possession of specific genes or chromosomes, rather than the ploidy. When reproducing sexually, new combinations of genes and chromosomes can be generated through several processes: (1) recombination between genes through crossing-over between chromosomes, (2) random assortment of chromosomes in gametes during meiosis, and (3) fusion of two different haploid genomes contained in gametes of two parents (egg and sperm). If, in a diploid cell, two identical copies of a gene are present, the condition is termed homozygous, whereas the occurrence of two different copies is termed heterozygous. Hemizygous refers to a situation in a diploid organism where only one copy of a chromosome or gene is present. This commonly occurs in the heterozygotic sex (i.e., the sex in which the sex chromosomes are different), for example an XY male is said to be hemizygous for almost all genes on the X chromosome. It also occurs where a copy of a gene has been deleted. Different versions of genes are known as alleles. In a heterozygous cell, a dominant allele is often expressed but the recessive allele may not be. Recessive alleles may only lead to a phenotype in the homozygous state. Dominant and recessive states are the extremes of a continuum and many genes are at least partly co-dominant, meaning both alleles may be expressed. In addition, gene expression may vary by tissue, life-stage and according to interactions with other genes and gene products, such that 'dominance' of an allele will vary within individual organisms (Sect. 3.5.2).

Most insects reproduce sexually, i.e., the haploid male gamete fuses with the haploid female gamete. There are two sexes, males and females, that produce sperm and eggs, respectively, through meiosis. Hermaphroditism, i.e., the male and female function combined in a single individual, is rare. Another common mode of reproduction in insects is parthenogenesis. Such asexual reproduction involves development of females from unfertilised eggs. Parthenogenetic reproduction has been found among insect natural enemy groups such as thrips (Thysanoptera), several non-parasitoid flies (Diptera), beetles (Coleoptera) and many Hymenoptera. In flies and beetles, parthenogenesis is frequently associated with polyploidy (Suomalainen et al., 1987).

The different reproductive modes which exist for insects have important consequences for the way in which traits are inherited. Whereas sexual reproduction leads to genetically heterogeneous offspring, certain forms of parthenogenesis lead to completely homozygous and hemizygous progeny within a single generation. Therefore, genetic knowledge of the reproductive modes of species is necessary for interpreting the genetic basis of variation in their life-history traits. In

addition, different selective pressures operating at each stage of the life-cycle can impact on the variants that persist and spread.

Many natural enemies of insects are economically important as agents of biological pest control. Variation in genetically controlled traits is a key factor affecting success, or failure of biological control. Hence knowledge of the genetic basis of traits is indispensable to biocontrol practitioners. Beneficial traits may deteriorate rapidly or be lost through inbreeding when animals are maintained in culture, particularly where there are high levels of inbreeding. However, optimised culturing techniques and selection experiments may improve natural enemy efficiency. For many traits including elements of morphology (e.g., wing-size); colour-patterns; physiology (e.g., the ability to escape the host's defence mechanism); biochemistry (e.g., insecticide resistance) and behaviour (e.g., activity patterns and mating preferences), a single gene (or a small number of major genes) has been shown to be responsible. These results have typically been obtained from controlled genetic crosses and demonstrate simple Mendelian genetics. Current molecular genetic techniques (e.g., cloning; whole genome sequencing; RAD-Seq; DArT Seq and other forms of genotyping-by-sequencing (GBS) and genetic manipulation-based technologies, including the latest genome editing (GE) techniques, such as CRISPR-Cas9), make it possible to isolate, characterise and modify such genes. However, many other traits, such as life-history traits, appear to be coded by many genes each having a small effect (polygenic control). Here, identification of genes, pathways and control regions may be highly complex. The study of such traits, therefore, has generally been limited to the field of quantitative genetics, an area that has progressed rapidly through development of new molecular and computational techniques.

It has been known for several decades that microbes and selfish genetic elements play a key role in some arthropod life-history traits, for example by manipulating reproductive behaviour and outcomes of insects. Recently, studies have begun to identify the ubiquity and variety of the microbiome across insect species and their natural enemies and the important role that these organisms play in shaping the fate of their hosts. Dicke et al. (2020) review microbial symbionts of parasitoids. Kageyama et al. (2012) and Ma et al. (2014) review endosymbiont manipulation of insect sex determination.

In this chapter we present details of available genetic data of insect natural enemies with emphasis on new research approaches and techniques.

3.2 Genetic Markers and Maps

3.2.1 Introduction to Markers

Genetic markers are indicators which show polymorphism within individuals or populations that can be used to monitor the inheritance of loci in the genome and the associated genes. They are indispensable in the study of population genetics. Genetic structures of populations were historically studied using enzyme electrophoresis. However, this technique is limited in the level of variation that can be detected. Progress in molecular biology, in particular the development of the polymerase chain reaction (PCR), led to the frequent use of more sensitive and abundant DNA techniques, such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) and more recently DNA fingerprinting, minisatellites and microsatellites. Techniques used for DNA and RNA sequencing have also developed rapidly, from radiation-based approaches in the 1970s, to ABI, fluorescently labelled Sanger sequencing, which has predominated for much of the last 40 years. Most recently, next-generation sequencing (NGS) technologies and applications have revolutionised the speed, cost and level of information that researchers can glean from samples. Initially using short-read technologies, the most recent single-molecule

techniques produce long reads, simplifying genome assembly and the creation of linkage maps, although accuracy of the sequence currently depends on the method used. In particular, the development of RNA sequencing methods (RNA-Seq) and genotyping by sequencing (GBS) mean that it is possible to sample any population for polymorphic markers and genetic structure, without needing existing genetic data to work from. Genetic sequence data is now widely used to identify organisms, transforming classical taxonomy and facilitating understanding of evolutionary relationships and ecological networks.

Here, we will briefly mention the main techniques currently used and refer to other works for more historical details of the methods and data analysis that have been used previously. Applications of the techniques will be discussed throughout the chapter if appropriate and some are also discussed in Chaps. 5 and 6. Practical guidelines for allozyme electrophoresis are given by Avise (1994), May (1992), and Steiner (1988). A more extensive overview of molecular genetic techniques and applications in insects is given by Hoy (1994). Practical guides for DNA techniques include Diefenbach and Dveksler (1995) and Sambrook et al. (1989). Books concerned with methods for analysing molecular and population data are those of Hartl and Clark (1989), Hoelzel (1992), and Weir (1990). Principles of genetic mapping are dealt with by Primrose (1995). There are many articles describing uses of the techniques. The following provide useful overviews: Davey et al. (2011) review genome-wide marker discovery and typing using next-generation sequencing; van Dijk et al. (2014) review next-generation sequencing; Heather and Chain (2016) review the history of DNA sequencing; van Dijk et al. (2018) review third-generation sequencing; Bak et al. (2018) review gene editing; DeSalle and Goldstein (2019) review an explosion of publications using DNA barcoding between 2004 and 2018, and Miller et al. (2021) explain DNA (meta)barcoding and describe uses in studying host-parasitoid networks, particularly within agricultural ecosystems.

3.2.2 Genetic Markers

Protein Markers

Allozymes

Enzyme electrophoresis is based on the separation of charged protein molecules in an electric field. The migration rate of a protein is not only related to its charge, but also to its size and shape. If the protein products of two alleles have different charges, their rates of migration will be different. This will be visible on a gel as separate bands after staining. Such allelic forms of the same locus are called allozymes. Most proteins examined are enzymes that catalyse specific biochemical reactions. They can consist of one single polypeptide unit (=monomer), two units (=dimer) or multiple units (=polymer). In addition, there can be more than one locus coding for the enzyme. Figure 3.1 shows the possible banding patterns of one-locus monomeric and dimeric enzymes in the homozygous or heterozygous state. More complicated patterns are explained in May (1992).

The process of enzyme electrophoresis can be divided into five steps (May, 1992): (1) extraction of proteins from tissue samples which should be frozen at −70 °C since most enzymes degrade easily; (2) separation of allozymes in an electric field, which is applied in a starch or polyacrylamide gel and appropriate buffer; (3) *staining* of the enzymes with specific substrates and dyes; (4) interpretation of the observed banding patterns (Fig. 3.1), and (5) application of the data to the specific research question.

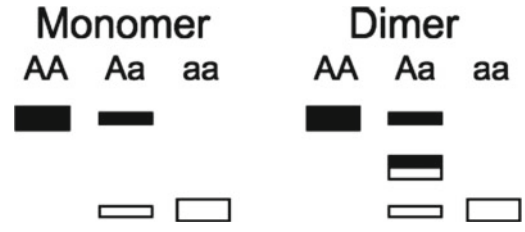


Fig. 3.1 Possible allozyme banding patterns for one locus with two alleles (A, a). Both homozygotes and the heterozygote for a monomeric and dimeric enzyme are shown

A number of parameters can be used to quantify the observed variation. The degree of polymorphism (*P*) is the proportion of loci at which multiple bands are found. The degree of heterozygosity (*H*) is the proportion of loci that are heterozygous. The genetic distance (*D*) is a measure of gene diversity between populations that is estimated using genotype frequencies (Hoelzel & Bancroft, 1992).

Previously, allozymes were used extensively in the study of insect natural enemies but have been largely superseded by DNA-based techniques. Unruh et al. (1986) summarised measures of genetic variation for 66 species of Hymenoptera and found that they maintain only about one-third of the level of heterozygosity of diploid insects ($H = 0.037$ vs. 0.120). Parasitoid wasps were not found to be different from other Hymenoptera. Enzyme electrophoresis is potentially still a useful technique for detecting endoparasitoids, measuring degrees of population differentiation and for distinguishing between strains or species, as it is relatively simple and inexpensive. However, it is limited by the number of polymorphic isozyme systems available.

Molecular Markers

Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphisms are obtained by digestion of DNA with restriction endonucleases that cut DNA at specific recognition sequences (usually 4–6 base pairs in length). Fragments are visualised through standard DNA electrophoresis, blotting and probing. The technique requires large amounts of DNA, although it can also be used together with PCR amplification (e.g., Vanlerberghe-Masutti, 1994). However, it has largely been superseded by faster methods which require less DNA.

Random Amplified Polymorphic DNA (RAPD)

The random amplified polymorphic DNA technique (Williams et al., 1990) is a DNA polymorphism assay based on the PCR amplification of random DNA segments with single, short primers. Primers are short oligonucleotides that can attach to a single stranded DNA molecule to initiate DNA replication. The RAPD technique relies on the statistical chance that two complementary primer sites occur in the genome as inverted repeats enclosing a relatively short stretch of DNA (up to a few thousand base pairs). RAPD primers are short (generally around ten nucleotides, i.e., decamers) and their nucleotide sequences are arbitrarily chosen. This technique detects DNA polymorphisms in the absence of specific nucleotide sequence information for the study species. RAPD bands are inherited as dominant traits, so heterozygotes cannot be identified readily. Specific advantages of the RAPD technique are its simplicity and rapidity: (1) only small amounts of DNA are required; (2) a universal set of primers can be used for many species; (3) no preliminary sequence information is needed; and (4) the number of primers is virtually unlimited, making detection of genetic polymorphism in even highly monomorphic populations possible. Its main drawback is its low reliability. Virtually all reaction steps and components can affect its reproducibility, including DNA extraction method, DNA and primer concentration, type of Taq polymerase and brand of thermocycler. As a pre-screen of polymorphism within a population, it may still have some value.

Amplified Fragment Length Polymorphism (AFLP)

The amplified fragment length polymorphism technique (Vos et al., 1995) detects variation in the presence of genomic restriction fragments. It is based on restriction digests of total genomic DNA and PCR amplification of a subset of restriction fragments to give a genomic representation and can as such be considered as a hybrid between the RFLP and RAPD techniques. It also combines the advantages of both techniques. It requires only small amounts of DNA and no previous information about DNA sequences. Nevertheless, it is much more reliable than the RAPD technique because it is based on DNA restriction endonuclease digests. DNA is digested with two enzymes, typically *Eco*RI and

*Mse*I, which generates small DNA fragments (<1 kb). Fragments are ligated (by formation of a phosphodiester bond using the enzyme ligase) to common adapter sequences (short synthetic oligonucleotides) which serve as primer binding sites. Genomic fragments are amplified in two consecutive PCR reactions, a pre-amplification round using primers with one random selective base to amplify 25% of the fragments, and a second selective amplification using primers with two or three selective nucleotides. The *Eco*RI selective primer is labelled and the PCR products visualised after electrophoresis, either on polyacrylamide gels or more commonly on a capillary sequencer. The AFLP technique is powerful because it can detect many different bands with a single reaction. Moreover, varying the number and nature of the selective bases can control the number of bands and sample different subfractions of the genomic enrichment. Although the AFLP technique was developed for plants, it appears equally useful for animals (e.g., Schneider et al., 2002). The same basic approach is used for genotyping by sequencing (GBS), but with the entire pool of fragments in the genomic representation being sequenced using NGS.

Minisatellites

Minisatellites consist of arrays of repeated 15–60 bp sequences which differ in copy number and are scattered throughout the chromosomes of many organisms. They are used for multi-locus DNA fingerprinting. They can be visualised using sequence-specific probes in Southern blot assays after restriction digestion of total genomic DNA (Bruford et al., 1992). Many tandem repeat units contain a common core sequence in different organisms which makes it possible to use similar probes in different insect species. DNA fingerprinting based on minisatellites can be used to identify individual insects or their progeny, as well as for parentage analysis. The absence of knowledge about which bands belong to which loci makes the technique less convenient for estimating population genetic parameters such as genetic relatedness. For the most part, minisatellite investigation has largely been superseded by microsatellites and sequence-based approaches.

Microsatellites

Microsatellites are short DNA sequences of 2–8 base pairs that occur as tandem arrays of several to hundreds of repeats in the genome of many organisms. They are among the most variable DNA sequences and are used extensively for DNA fingerprinting (Bruford et al., 1992). They can be visualised with standard PCR and electrophoresis techniques using primers that are specific to the sequences of the flanking regions. Their main drawback has been the large effort involved in obtaining such sequence information. This used to require construction of a genomic library, screening of clones for repeat sequences and subsequent sequencing of clones.

With the advent of NGS, and particularly RNA-Seq, it has become possible to screen the genome or transcriptome of organisms for microsatellite repeats. This has the advantage of large-scale *in silico* detection of microsatellite repeats. For microsatellites located in the expressed genome, these come from low-copy regions of the genome. If multiple individuals are sequence surveyed, then it may also be possible to detect whether a particular locus is polymorphic within the population of interest *in silico*. Microsatellites are highly reliable and usually locus specific, so for fingerprinting and analysis of transmission and survival, they can be used to screen large numbers of individuals (e.g., Khidr et al., 2013, 2014). However, where a broad survey of the entire genome is needed, techniques such as RAD sequencing (Miller et al., 2007; Baird et al., 2008) or genotyping by sequencing (Elshire et al., 2011) are used. Scheben et al. (2017) provide a useful review of GBS, focused on crop genome characterisation. Microsatellite-based fingerprinting has been the most reliable method for identification of progeny and paternity analysis (e.g., Guo et al., 2022), but the development of NGS does allow alternative approaches, which may be less polymorphic per locus, but produce single nucleotide polymorphism (SNP) data across thousands of loci.

DNA Sequencing

DNA sequencing yields information about variation at the nucleotide level. Historically, sequencing was both expensive and time-consuming, hence the fraction of the genome analysed was limited. Which part of the genome was sequenced was also strongly dependent on the research question posed. Typically, DNA sequencing was applied in studies of variation at a higher level than the individual, with some regions such as ribosomal DNA and the mitochondrial cytochrome genes being used frequently in phylogenetic analyses, intended to separate strains or species (e.g., Field et al., 1988; Gimeno et al., 1997). Such DNA sequences of specific regions are now routinely used to characterise and identify organisms where phenotypic characterisation and/or rearing is difficult or impossible. The most frequently used regions are the 16S rRNA gene, to identify bacteria (Woese & Fox, 1977; Weisburg et al., 1991) and the mitochondrial cytochrome c oxidase I (COI) gene, in what are now known as DNA barcodes (Hebert et al., 2003). Of particular relevance to natural enemies of insects, investigation of 16S rDNA has been widely used to identify bacterial symbionts, such as those involved in reproductive manipulation (Sects. 3.4.2 and 6.5.1) and resistance to viruses (Sect. 6.5.3), whilst DNA barcoding and metabarcoding (parallel amplification and sequencing of multiple organisms from a single sample) are becoming increasingly useful tools in understanding ecological networks and host–parasitoid relationships (Miller et al., 2021).

Sequencing cost, time taken, plus the amount of material required to obtain useful sequence reliably, have all decreased markedly over recent decades. This has meant, increasingly, that sequence-based techniques have become the methods of choice for the majority of investigations that seek to understand the genetics and inheritance of variation across individuals and populations in the natural world. It is beyond the scope of this chapter to review all the iterations and variants of sequencing technologies. However, as starting points for further information, see DeSalle and Goldstein (2019), van Dijk et al. (2014, 2018), Heather and Chain (2016), Hess et al. (2020), Shendure et al. (2017), and Slatko et al. (2018); plus see Jung et al. (2020) for a review of the approaches and bioinformatic pipelines used to assemble and annotate genomic sequencing data. Key to investigations of insects and insect enemies, so-called second- and third-generation NGS techniques, are currently the most widely used.

NGS Techniques

The advent of next-generation sequencing and associated approaches facilitated a step-change in the ability to investigate questions in ecology, population and conservation genetics and evolution. These approaches make use of the thousands of polymorphic markers present in the genomes of every organism that one might wish to study. They are enabling detailed investigation of quantitative trait loci (QTL) (Sect. 3.5), phenotypic trait inheritance and phylogeography. They are supporting marker-assisted selection of novel and beneficial variants. The speed with which data are generated has increased rapidly over recent years and bioinformatic methods are expanding to facilitate analysis and informative visualisation of the vast amounts of data produced in many studies. Applications can range from sequencing and assembling an entire insect genome and surveying other individuals at lower depth, to capture-and-sequence techniques which focus on specific groups of loci. For deep sampling, RAD-Seq and GBS techniques are ideal. For large numbers of individuals at reasonable cost, the development of sets of polymorphic markers applying competitive allele-specific PCR (KASP) (He et al., 2014) or similar approaches are ideal. Goodwin et al. (2016) review ten years of NGS technologies.

Genome Sequencing

The ability to generate large amounts of DNA sequencing has led to the rapid increase in the availability of complete and partial genome sequences for a wide range of species, including some parasitoids. The NCBI Whole Genome Sequence browser lists (as of 11-11-2021) a total

of 20,444 projects in eukaryotes (either complete or partial) comprising over 1500 different insects, including 225 hymenopteran species, often more than one per genus. For example, there are three entries for *Nasonia* species (*N. vitripennis*, *N. giraulti* and *N. longicornis*) (Werren et al., 2010). More recently, genome sequences for *Microplitis demonliter* (Burke et al., 2018b), *Trichogramma pretiosum* (Lindsey et al., 2018) and *Megaphragma amalphitanum* (Sharko et al., 2019) have been reported.

The existence of a genome sequence allows a wide range of questions to be asked about parasitoid population and genome biology as well as helping to confirm relationships in the wild, for example barcoding of parasitoid wasps associated with wild and cultivated olives in South Africa (Powell et al., 2019). Methylation of DNA has long been known to be important for control of gene expression, suppression of mobile genetic elements and, more recently, for a potential role in adaptation. Cook et al. (2018) examined the effects of using a demethylation agent on sex allocation and other traits in *Nasonia vitripennis*. The same basic approach can also be used to investigate chromosome biology and evolution, such as recombination patterns (Niehuis et al., 2010), parasitoid endosymbionts (Lindsey et al., 2016), mitochondrial changes (Chen et al., 2018), viral interactions (Burke et al., 2018a) and host protection (Hansen et al., 2012). Having genome sequencing for the host, the parasitoid and even endosymbionts and hyperparasitoids will allow a better understanding of the complex interactions between all of the organisms involved in the relationship.

Phenotypic Markers

Phenotypic markers (or visible mutations) have traditionally been used in genetic studies of insects. The most abundant ones are eye-colour, body-colour and other morphological mutations. Eye-colour and body-colour mutations are known from many parasitoid wasps (e.g., Whiting, 1932, 1934, 1954; Baldwin et al., 1964; Saul et al., 1965; McInnis et al., 1986). Examples of morphological mutants include a wingless mutant in the two-spot ladybird *Adalia bipunctata*, which lacks all or part of the elytra and flight wings (Marples et al., 1993) and several "vestigial", "shrivelled" and "stubby" wing and "club" antennal mutants in parasitoid wasps (Whiting, 1935; Baldwin et al., 1964). Saul et al. (1965) described mutants for each of five linkage groups in the chalcidoid wasp *Nasonia vitripennis*. Most of the stocks of these mutants are now maintained in Rochester (USA) and Groningen (The Netherlands).

Mutants are generated by X-rays or chemical mutagenesis, such as ethylmethanesulfonate (EMS), administered during larval or adult stages. Protocols developed for *Drosophila* (Grigliatti, 1986) can readily be used for other organisms. Detailed information on dose–response curves of X-rays in *Nasonia* are given by Kayhart (1956) and Whiting (1967). They obtained eye-colour mutants with a frequency of up to 1%. Mutations are typically recessive to wildtype alleles. Some are female sterile and less viable in the homozygous state, requiring specific crosses and propagation through heterozygous females. In haplodiploids, they can easily be detected in haploid males (see below).

3.2.3 Investigating Inheritance and Genetic Control

One-Point Cross

The simplest genetic organisation of a trait is that it is coded by a single gene. Examples are most eye-colour mutants, which are caused by recessive mutations in single genes. A first approach to reveal the genetic basis of a trait is to cross two individuals that carry different forms of the trait. These individuals are referred to as the parental generation (P). The offspring of such a cross are referred to as the first filial generation, or F_1, a second generation as F_2 and so on. Figure 3.2a shows an example of the inheritance of a single-gene trait, vestigial wings, in a diploid organism. The wildtype (i.e., 'normal, non-mutant') form is dominant and therefore F_1 heterozygotes have wildtype wings. In diploids, crossing of two heterozygous F_1 individuals yields homozygous

wildtype, heterozygous wildtype and homozygous vestigial winged F_2 individuals at frequencies of 1:2:1, which in this case translates into phenotypic ratios of 3 wildtype:1 vestigial.

As mentioned in Sect. 3.1.1, and described in Sect. 3.3, many insects are haplodiploid (males are haploid and females are diploid). In haplodiploids, F_1 females can reproduce unmated (virgin), resulting in haploid male offspring with wildtype and vestigial wings in a ratio of 1:1. In haploids, there is no dominance/recessiveness, and genotypic and phenotypic ratios are equal. Figure 3.2b shows the inheritance of the vestigial trait in a haplodiploid organism.

An illustrative example of how to detect a recessive trait controlled by a single gene is the wingless morph in the diploid two-spot ladybird, *Adalia bipunctata*, studied by Marples et al. (1993). These authors found a male with the elytra completely absent and the flight wings reduced to small buds. They mated it to a normal winged female. The twelve resulting F_1 offspring were all fully winged. This observation is consistent with a trait controlled by a single gene. However, it cannot yet exclude multiple genes, or that the wingless individual had simply been damaged. Therefore, the authors mated F_1 individuals among themselves and obtained fourteen wingless and 34 winged F_2 offspring, which is consistent with the 1:3 ratio expected for a single recessive allele.

Two-Point Cross

Figure 3.3 shows an example of the inheritance of two single-gene traits, vestigial wings and ebony eye-colour with independent assortment (i.e., the genes are inherited independently of each other, rather than linked as described in the three-point cross). Both mutant alleles are recessive, as in the one-point cross above. The test cross, which is typically used in gene-mapping studies (Sect. 3.2.4), involves mating the F_1 offspring to one of the parental lines in the diploid case (Fig. 3.3a) and allowing virgin reproduction in the haplodiploid case (Fig. 3.3b). Four types of offspring are produced in equal

a

	Diploid			**Haplodiploid**		
Parental strains	male	x	female	male	x	female
Genotype	*vg / vg*	x	+ / +	*vg*		+ / +
Phenotype	*vestigial*		wildtype	*vestigial*		wildtype
Gametes	*vg*		+	*vg*		+
F1 progeny						
Genotype	*vg / +*			*vg / +*		
Phenotype	wildtype			wildtype		
Test cross	F_1	x	F_1	virgin female		
Genotype	*vg / +*		*vg / +*	*vg / +*		
Gametes	+		+	+		
	vg		*vg*	*vg*		
F2-progeny	males + females			males only		
Genotype	+ / +	+ / *vg*	*vg / vg*	+		*vg*
Phenotype	wildtype	wildtype	*vestigial*	wildtype		*vestigial*
Expected ratio	1	2	1	1		1

Fig. 3.2 Example of a one-point cross for a diploid (**a**) and haplodiploid (**b**) organism. A cross is performed between a male with mutant vestigial wings and a female with normal wildtype wings. The wildtype allele is denoted by +. The mutant vestigial allele, denoted by *vg*, is recessive, hence all F_1 offspring are genotypically heterozygous but phenotypically wildtype. In the diploid (**a**), the F_1 are crossed among themselves. The F_2 offspring consist of three genotypic classes in proportions 1:2:1 (i.e., homozygous +, heterozygous + *vg* and homozygous *vg* respectively), but only two phenotypic classes in proportions 3:1 (i.e., wildtype and vestigial respectively). In the haplodiploid (**b**), the F_1 virgin female produces haploid wildtype and vestigial sons in equal ratio

numbers under both reproductive modes, i.e., each combination of the two traits.

Many quantitative traits are coded for not by a single gene but by several genes. Figure 3.4 shows the inheritance of a wing-size trait that is based on two genes. The assortment of genes is similar to that shown in Fig. 3.3, but the resultant phenotypes are different. This is caused by the fact that alleles at both loci are additive, i.e., their effects add up (see also Fig. 3.18). Homozygous wildtype ("l" form) individuals have long wings whereas individuals homozygous for the "s" form have short wings. The heterozygous F_1 offspring have wings of intermediate length (one "l" allele and one "s" allele at each locus). In the diploid case (Fig. 3.4a), crossing two heterozygous F_1 individuals yields three types of intermediate forms in the F_2 depending on what proportion of their alleles are of the "s" form: 75% short (3 "s" alleles), intermediate (2 "s" alleles) and 25% short (1 "s" allele) in addition to the parental short (4 "s" alleles) and long (zero "s" alleles, 4 "l" alleles) phenotypes. There are different combinations of alleles (i.e., genotypes) that create the various phenotypic forms (for example the 75% short form can be s_1s_2/s_1l_2; s_1s_2/l_1s_2; l_1s_2/s_1s_2 or s_1l_2 s_1s_2). In this example, the five possible phenotypes (short, 75% short, intermediate, 25% short and long) are produced in the ratio 1:4:6:4:1. Note that when genes are additive, the F_2 ratios are informative about the number of genes that underlie a certain trait (Roff, 1997).

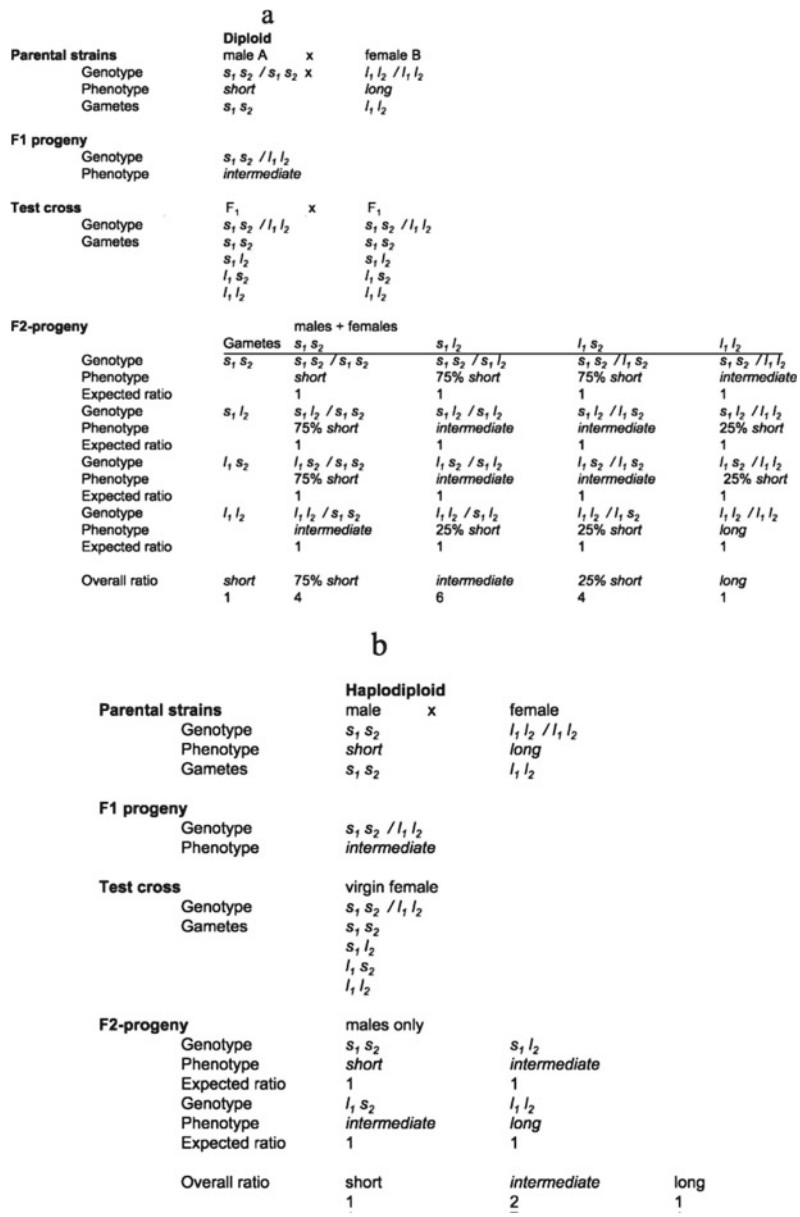
In the haplodiploid case (Fig. 3.4b), one can breed from a single unmated female. This results in males with three possible phenotypes (short, intermediate and long) in a ratio of 1:2:1. Figure 3.5 shows the wing-size distributions

a

	Diploid		Haplodiploid	
Parental strains	male A x	female B	male x	female
Genotype	*vg e / vg e* x	+ + / + +	*vg e*	+ + / + +
Phenotype	*vestigial ebony*	wildtype	*vestigial ebony*	wildtype
Gametes	*vg e*	+ +	*vg e*	+ +
F1 progeny				
Genotype	*vg e* / + +		*vg e* / + +	
Phenotype	wildtype		wildtype	
Test cross	F_1 x	A virgin female		
Genotype	*vg e* / + +	*vg e / vg e*	*vg e* / + +	
Gametes	+ +	vg e	+ +	
	vg +		*vg* +	
	+ *e*		+ *e*	
	vg e		*vg e*	
F2-progeny	males + females		males only	
Genotype	+ + / *vg e*	*vg* + / *vg e*	+ +	*vg* +
Phenotype	wildtype	*vestigial*	wildtype	*vestigial*
Expected ratio	1	1	1	1
Genotype	+ *e* / *vg e*	*vg e / vg e*	+ *e*	*vg e*
Phenotype	*ebony*	*vestigial ebony*	*ebony*	*vestigial ebony*
Expected ratio	1	1	1	1

b

Fig. 3.3 Example of a two-point cross for a diploid (**a**) and haplodiploid (**b**) organism. A cross between a male of strain A with mutant vestigial (*vg*) wings and ebony (*e*) body-colour, and a female of strain B with normal wildtype wings and body-colour. The mutant vestigial and ebony alleles are recessive, hence all F_1 offspring are genotypically heterozygous and phenotypically wildtype. In the diploid (**a**), the F_1 are back-crossed to mutant strain A. The genes must be located on different chromosomes because the four possible F_2 genotypes and phenotypes occur in a 1:1:1:1 ratio. In the haplodiploid (**b**) the F_1 females are bred as virgins and the F_2 males have four different genotypes and phenotypes in proportions 1:1:1:1



a

Diploid			
Parental strains	male A	x	female B
Genotype	$s_1\,s_2\,/\,s_1\,s_2$	x	$l_1\,l_2\,/\,l_1\,l_2$
Phenotype	*short*		*long*
Gametes	$s_1\,s_2$		$l_1\,l_2$
F1 progeny			
Genotype	$s_1\,s_2\,/\,l_1\,l_2$		
Phenotype	*intermediate*		
Test cross	F_1	x	F_1
Genotype	$s_1\,s_2\,/\,l_1\,l_2$		$s_1\,s_2\,/\,l_1\,l_2$
Gametes	$s_1\,s_2$		$s_1\,s_2$
	$s_1\,l_2$		$s_1\,l_2$
	$l_1\,s_2$		$l_1\,s_2$
	$l_1\,l_2$		$l_1\,l_2$

F2-progeny — males + females

Gametes	$s_1\,s_2$	$s_1\,l_2$	$l_1\,s_2$	$l_1\,l_2$
$s_1\,s_2$	$s_1\,s_2\,/\,s_1\,s_2$	$s_1\,s_2\,/\,s_1\,l_2$	$s_1\,s_2\,/\,l_1\,s_2$	$s_1\,s_2\,/\,l_1\,l_2$
Phenotype	*short*	75% *short*	75% *short*	*intermediate*
Expected ratio	1	1	1	1
$s_1\,l_2$	$s_1\,l_2\,/\,s_1\,s_2$	$s_1\,l_2\,/\,s_1\,l_2$	$s_1\,l_2\,/\,l_1\,s_2$	$s_1\,l_2\,/\,l_1\,l_2$
Phenotype	75% *short*	*intermediate*	*intermediate*	25% *short*
Expected ratio	1	1	1	1
$l_1\,s_2$	$l_1\,s_2\,/\,s_1\,s_2$	$l_1\,s_2\,/\,s_1\,l_2$	$l_1\,s_2\,/\,l_1\,s_2$	$l_1\,s_2\,/\,l_1\,l_2$
Phenotype	75% *short*	*intermediate*	*intermediate*	25% *short*
Expected ratio	1	1	1	1
$l_1\,l_2$	$l_1\,l_2\,/\,s_1\,s_2$	$l_1\,l_2\,/\,s_1\,l_2$	$l_1\,l_2\,/\,l_1\,s_2$	$l_1\,l_2\,/\,l_1\,l_2$
Phenotype	*intermediate*	25% *short*	25% *short*	*long*
Expected ratio	1	1	1	1
Overall ratio	*short*	75% *short*	*intermediate*	25% *short*	*long*
	1	4	6	4	1

b

	Haplodiploid		
Parental strains	male	x	female
Genotype	$s_1\,s_2$		$l_1\,l_2\,/\,l_1\,l_2$
Phenotype	*short*		*long*
Gametes	$s_1\,s_2$		$l_1\,l_2$
F1 progeny			
Genotype	$s_1\,s_2\,/\,l_1\,l_2$		
Phenotype	*intermediate*		
Test cross	virgin female		
Genotype	$s_1\,s_2\,/\,l_1\,l_2$		
Gametes	$s_1\,s_2$		
	$s_1\,l_2$		
	$l_1\,s_2$		
	$l_1\,l_2$		

F2-progeny — males only

Genotype	$s_1\,s_2$		$s_1\,l_2$	
Phenotype	*short*		*intermediate*	
Expected ratio	1		1	
Genotype	$l_1\,s_2$		$l_1\,l_2$	
Phenotype	*intermediate*		*long*	
Expected ratio	1		1	
Overall ratio	short		*intermediate*	long
	1		2	1

Fig. 3.4 (**a**) Example of the inheritance of a trait that is coded for by two unlinked genes in a diploid organism. A cross between a male of strain A with short (s_1 and s_2) wings and a female of strain B with long (l_1 and l_2) wings. The short and long alleles are co-dominant, hence all F_1 offspring are genotypically heterozygous and have intermediate wings. In the diploid (**a**), the F_1 are crossed among themselves. There are sixteen possible genotypes and five phenotypes in proportions 1:4:6:4:1. (**b**) Example of the inheritance of a trait that is coded for by two unlinked genes in a haplodiploid organism. A cross between a male of strain A with short (s_1 and s_2) wings and a female of strain B with long (l_1 and l_2) wings. The short and long alleles are co-dominant, hence all F_1 offspring are genotypically heterozygous and have intermediate wings. In the haplodiploid (**b**) the F_1 females are bred as virgins and the F_2 males have four different genotypes and three phenotypes in proportions 1:2:1

(corrected for body-size) of parents and F_2 males of a cross between two haplodiploid *Nasonia* sibling species, the short-winged *N. vitripennis* and the long-winged *N. giraulti* (Weston et al., 1999). The F_2 distribution is intermediate to the parental ones, but still clearly bimodal. The 44% wing-size variation in these species can be explained by a single major gene, but there are also several genes with minor effect (Sect. 3.5.4).

Three-Point Cross

Thus far, we have considered genes inherited independently from each other. However, genes that are located on the same chromosome may be linked and frequently segregate together. Only through the formation of cross-overs between genes on a single chromosome may such genes be uncoupled. Therefore, the frequency at which two linked genes are map distances inherited

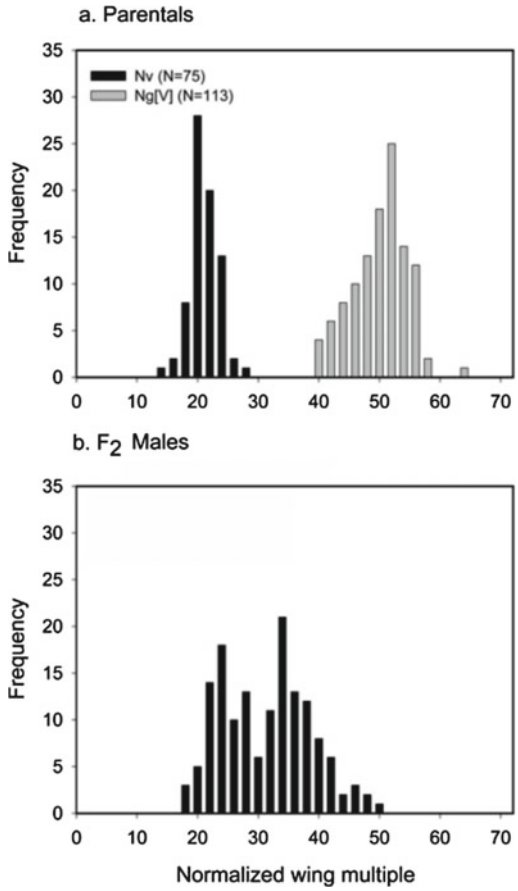


Fig. 3.5 The distribution of forewing sizes (corrected for body-size) of parents and F_2 males of a cross between two haplodiploid *Nasonia* sibling species, the short-winged *N. vitripennis* (Nv) and the long-winged *N. giraulti* (Ng[V]). The F_2 distribution is intermediate to the parental ones and bimodal, indicating a single gene with major effect and one or more genes with small effect. *Source* Weston et al. (1999). Reproduced by permission of Blackwell Publishing

independently is used as a measure for recombination. Recombination frequencies, in turn, are informative about the physical distance between genes (so-called map distances). Figure 3.6 shows an example of how to estimate recombination frequencies and establish map distances in a cross with three linked genes in *Drosophila*.

Iso-female Lines

A powerful way of demonstrating the presence of genetic variation for a trait is through iso-female lines (Parsons, 1980). An iso-female line is a laboratory strain that is bred from a single mated female. In haplodiploid species, such as parasitoid wasps, a strain can be started from a single female if she can be kept alive long enough to mate with one of her sons. Typically, an array of lines is established from field-collected animals, and they are scored for a trait under identical laboratory conditions. The between-line variance component is a measure for the amount of genetic variation for the trait. One drawback of this method is that it results in inbreeding which can have a number of negative effects on fitness, particularly in diploid species. This is especially a problem in life-history research because inbreeding tends to cause spurious positive correlations between sets of traits (the best iso-female line is best in most traits). A second drawback is the fact that the estimate of genetic variation obtained from iso-female line comparisons does not distinguish between additive and dominance genetic variation (Sect. 3.5).

Artificial Selection

Another valuable genetic tool is artificial selection. Artificial selection can take place in populations in which individual variation has at least partly a genetic basis and by selectively breeding those individuals with the most extreme phenotype for the trait. For example, in a population of differently sized individuals, the largest can be chosen each time as parents to found the next-generation. As generations pass, the selected line will become enriched for alleles that lead to larger size, and the average size of individuals will increase. Simultaneously, selection can take place for smaller size by breeding the smallest individuals each generation. If selection is carried on for long enough, two sub-populations may result that have non-overlapping body sizes. The responses to selection can be used to estimate variance components and heritabilities for the selected and correlated traits (Sect. 3.5.3). Both the iso-female line and artificial selection techniques are further discussed in the context of genetic variation for sex ratio (Sect. 3.4.2) and host–parasitoid interaction (Sect. 3.5.3).

		Diploid		
Parental strains		male A	x	female B
Genotypes		+ + + / Y		*vg e r / vg e r*
Phenotypes		wildtype		*vestigial ebony red*
Gametes		+ + +		*vg e r*
		Y		
F1 progeny				
Genotype		+ + + / *vg e r*		*Y / vg e r*
Phenotype		wildtype female		*vestigial ebony red* male
Test cross		F_1 female	x	B male (or F_1 brothers)
Genotype		+ + + / *vg e r*		*Y / vg e r*
Gametes	parental	+ + +		*vg e r*
		vg e r		Y
	single cross-over I	*vg* + +		
		+ *e r*		
	single cross over II	*vg e* +		
		+ + *r*		
	double cross over	vg + r		
		+ *e* +		

F2-progeny

Genotype		Phenotype Number	Class	Class total	Frequency	Recombination frequencies
males	females					
+ + + / Y	+ + + / *vg e r*	wildtype	250	parental		
vg e r / Y	*vg e r / vg e r*	*vestigial ebony red*	260	parental	510	
vg + + / Y	vg + + / *vg e r*	*vestigial*	38	single I		
+ *e r* / Y	+ *e r / vg e r*	*ebony red*	42	single I	80	*a* = 80 / 1000 = 0.080
vg e + / Y	*vg e* + / *vg e r*	*vestigial ebony*	205	single II		
+ + *r* / Y	+ + *r / vg e r*	*red*	195	single II	400	*b* = 400 / 1000 = 0.400
vg + *r* / Y	*vg* + *r / vg e r*	*vestigial red*	4	double		
+ *e* + / Y	+ *e* + / *vg e r*	*ebony*	6	double	10	*c* = 10 / 1000 = 0.010
			Grand total		1000	

Thus:

$$c_{vg,e} = (a + c) * 100 = 9.0$$
$$c_{e,r} = (b + c) * 100 = 41.0$$
$$c_{vg,r} = (a + b) * 100 = 48.0$$

Hence:

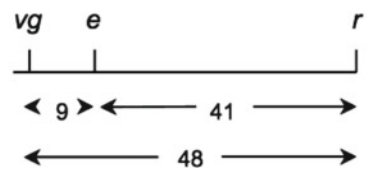


Fig. 3.6 Example of a three-point cross with sex chromosome linkage for a diploid organism. **Upper panel**: A cross between a male of strain A that is wildtype for three mutations that are X-linked and a triple mutant female of strain B with vestigial (*vg*) wings, ebony (*e*) body-colour and red (*r*) eyes. The mutant vestigial, ebony and red alleles are recessive, hence all F_1 offspring are genotypically heterozygous and phenotypically wild-type. The F_1 is either back-crossed to strain B or the F_1 females to their brothers. The F_2 is scored for their phenotype and it appears that the three genes are not segregating independently, but are linked. Eight different phenotypes can be distinguished that belong to four recombination classes. The class with the lowest numbers refers to double cross-overs and from this the order of the markers can be seen immediately as *vg e r* (i.e., to separate *e* from *vg* and *r* you would need two recombination events). **Lower panel**: The frequencies of the recombination classes give information on what the parental genotypes were. The recombination frequencies can be calculated and measure the relative distance between the markers expressed in centiMorgans. Note that the sum of $C_{vg,e}$ and $C_{e,r}$ is larger than $C_{vg,r}$. This happens as a result of double cross-overs (see text). For haplodiploids the results are similar to X-linkage in diploids, except that the Y chromosome is absent

3.2.4 Genetic Maps

A genetic map outlines the total genome in terms of number of chromosomes and the order of loci along these chromosomes. The loci serve as landmarks on a geographical map: they indicate the particular chromosome as well as the position of the gene or genetic marker on the chromosome. Constructing a linkage map originally took considerable effort, but with NGS-based approaches, such as RAD-Seq and GBS, it becomes possible to generate the large number of polymorphic loci needed to construct comprehensive genetic maps. However, the breeding system does have an effect on the overall levels of polymorphism and haplodiploid systems will have strong purifying selection through the haploid males and exposed recessive mutations, unless these mutations are neutral in effect. Apart from being valuable in providing a physical description of genome size and number of chromosomes for a species, genetic maps are essential for the mapping of (new) genes onto the genome. Such genes could be underpinning single locus traits (e.g., an eye-colour mutant) as well as polygenic traits (e.g., body-size). Most quantitative traits are polygenic, and the technique of localising polygenes is known as quantitative trait loci (QTL) mapping (Sect. 3.5.4). In addition, genetic maps can be used for marker-based introgression experiments (i.e., using genetic markers to aid the transfer of genes or traits from one strain to another) and for studying gene flow between natural or laboratory populations. Finally, an important use of genetic maps is to determine on which part of the genome natural selection is operating under field conditions (Wadgymar et al., 2017).

Historically, detailed maps were only available in a few well-studied species with short generation times, such as *Drosophila melanogaster*. The genetic markers were mostly morphological mutants. For instance, for *Drosophila*, several hundred such loci were described (Lindsley & Zimm, 1992), but fewer for the parasitoid wasps *Habrobracon hebetor* (Whiting, 1961) and *Nasonia vitripennis* (Saul et al., 1965). Although later cryptic protein variation such as allozymes became detectable, neither these nor the major mutants were sufficiently abundant or polymorphic. With the advent of DNA-based molecular markers the construction of genetic linkage maps became feasible in virtually all species. The wide availability and increasingly affordable suite of NGS technologies has meant that whole genome sequences are now also being assembled, not just for model organisms but for a wide and increasing variety of examples across all taxa.

Basic Principle of Mapping

In constructing a genetic map, both the ordering and the relative distance between loci on chromosomes is determined. The order of the loci and the relative distance between them can be established through the analysis of marker allele segregation in test and other controlled crosses, which reflects the frequency of recombinational exchange during meiosis. The basic procedure is outlined below.

In order to maximise the efficiency of map construction, the parental lines in the test crosses have to carry different alleles at each of the marker loci used, because then all markers will be informative. This is commonly the case for inbred laboratory stocks, or for populations from different geographical regions. Divergent selection lines can also be used, however depending on the number of generations of selection from a common ancestral stock, the number of informative markers may be limited. Once the choice of parental stocks is made, they are crossed and the resulting F_1 individuals are either backcrossed to both or one of the parents, or intercrossed, to produce the F_2 generation (Fig. 3.6). For diploid organisms, the back-cross design is most commonly used for dominant markers (e.g., presence or absence of RAPD markers) and the F_2 design for co-dominant markers (e.g., microsatellites). Since a large number of insect natural enemies are parasitoid wasps, it is noteworthy that, for linkage analysis in haplodiploid species, all meioses in the F_1 female are informative because recessive marker alleles can be scored (Antolin et al., 1996). For instance, in constructing a linkage map for *Habrobracon*

hebetor, the sexually derived virgin F_1 females were given abundant hosts (moth larvae) to produce all-male broods. These male individuals were then genotyped for the markers (Antolin et al., 1996).

Another way to ensure a high number of informative markers is to use single pair crosses and score the genotype (and when used in combination with gene-mapping approaches, the phenotype) of all the resulting progeny. In this case, even though particular markers are not fixed in the two parental lines, they may still be informative in a single pair cross. For instance, if the allele frequency of a marker gene is 0.9 for allele A_1 in parental line 1 and 0.1 in parental line 2, the chance of a $A_1A_1 \times A_2A_2$ cross is, $(0.9)^2(0.9)^2 = 0.656$. This makes even unfixed markers useful providing that enough markers are available. This approach has been successfully used in a genetic association study (Beldade et al., 2002). Coupled with NGS techniques for generating markers, this can allow the development of dense genetic maps, without the need to develop specific stocks for mapping.

Once the progeny from the crosses have been genotyped, the recombination frequency (RF) for pairs of markers can be calculated. To do this, knowing the parental genotypes for each of the markers is convenient. However, this is not essential because the two most frequent genotypes for any pair of markers can be considered the parental types. When calculating the recombination frequencies it will become apparent which markers form one linkage group and which lie outside this linkage group (i.e., respectively, $c < 50$ and $c = 50$ Morgan; Fig. 3.6). In principle, a linkage group represents one chromosome, and this can be confirmed with cytological data. Given that enough progeny have been scored so that the *c*-values are accurately calculated within each linkage group the order of the markers can be established unambiguously.

To construct a map based on a linear distance scale (expressed in centiMorgans, cM, where one cM represents a recombination frequency of 1%) the *c*-values (RF) have to be converted using a genetic mapping function. This is so because the *c*-values are not additive. Consider three linked loci A, B and C with recombination frequencies c_{AB}, c_{AC}, c_{BC}. The recombination frequency c_{AC} will be underestimated because recombination between B and C can mask the recombination between A and C in terms of the actual occurring genotype. This is the case when there is no interference, i.e., the presence of a cross-over between chromatids does not influence the likelihood of another cross-over occurring in the (close) vicinity. These aspects can be formulated as follows (Lynch & Walsh, 1998):

$$c_{AC} = c_{AB} + c_{BC} - 2(1 - \delta)c_{AB}c_{BC} \qquad (3.1)$$

with the interference parameter, δ, ranging between 0 (no interference) and 1 (complete interference). From (3.1) it follows that recombination values can only be considered additive when there is strong interference, or when the markers are closely linked (i.e., the fraction $2(1 - \delta)\ c_{AB}\ c_{BC}$ can be ignored). There are several mapping functions that predict physical distances from recombination frequencies, and we will mention the two most widely used.

Haldane's mapping function (Haldane, 1919) assumes random cross-over formation based on a Poisson distribution and no interference (Lynch & Walsh, 1998):

$$m = -\ln(1 - 2c_{AB})/2 \qquad (3.2)$$

with m being measured in Morgans or centiMorgans.

Kosambi's mapping function (Kosambi, 1944) permits some interference:

$$m = [\ln(1 + 2c_{AB})/(1 - 2c_{AB})]/4 \qquad (3.3)$$

For small recombination values ($c < 0.15$) the differences between Haldane's, Kosambi's or the $m = c$ mapping functions are negligible (Fig. 3.7) (Kearsey & Pooni, 1996; Lynch & Walsh, 1998).

Construction of Linkage Maps

It is obvious that constructing linkage maps by hand is cumbersome and near to impossible when the number of markers involved is high.

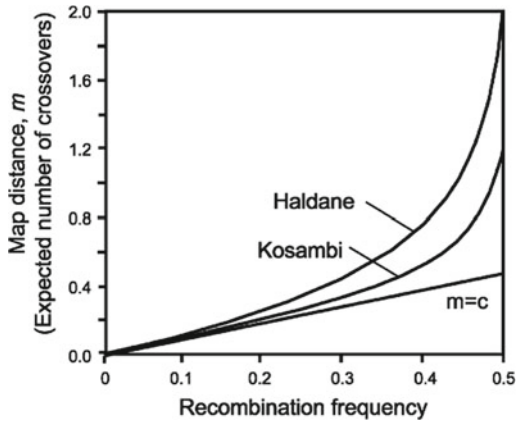


Fig. 3.7 The relationship between map distance (m) and recombination value (c) for three mapping functions, Haldane's (Haldane, 1919), Kosambi's (Kosambi, 1944) and $m = c$

Therefore, the use of computers is essential and several packages have been developed (Stam, 1993), of which MAP-MAKER (Lander et al., 1987), CRI-MAP (Lander & Green, 1987; Green et al., 1990) and JoinMap (Stam, 1993; https://www.kyazma.nl/index.php/JoinMap/General/) are the most widely used (more examples are provided at http://gaow.github.io/genetic-analysis-software/0/). The programs construct the most likely linkage map, both in terms of ordering of markers as well as the distance between the markers. They can use least-square procedures or the wider class of maximum likelihood procedures.

For instance, JoinMap first calculates recombination frequencies and LOD scores for pairs of markers. LOD stands for the logarithm of odds, that is, the logarithm of the ratio of the probability that the two markers are linked over the probability that they are unlinked (Stam, 1993). Thus, the higher the LOD score the more strongly two markers are linked. Secondly, linkage groups are established using a user-set LOD threshold value, below which markers are considered to be unlinked. A marker is judged on its LOD score to be significantly linked to any of the markers already added to the marker group. If the marker is not linked to any of the existing linkage groups, a new group is created. This process will stop when all markers have been added to the map groups. Depending on the threshold LOD value, a unique set of grouped markers will result. Generally, a threshold LOD of 3 works well to ensure that the number of linkage groups corresponds with the actual number of chromosomes. Individual groups of interlinked markers are then ordered, starting with the two markers with the highest LOD in the regression mapping approach, with further markers added until all are included from that group or have been rejected (Stam, 1993). The quality of the linkage map, first and foremost, depends on the quality of the data used: the map is only as good as the data. Large data sets and/or multiple estimates of the c-value for each pair of markers will greatly enhance the quality of the map.

Available Genetic Maps

Various techniques and marker types have been used to create linkage maps for insect natural enemies. Initially these were all for parasitoid wasps. The first linkage map was for *Habrobracon hebetor* with 79 RAPD-SSCP markers. It had a total length of 1156 cM and an average spacing of one marker per 17 cM (Antolin et al., 1996; Fig. 3.8). A RAPD marker-based linkage map was published for *Trichogramma brassicae*, with very similar features, 84 markers having an average spacing of 17.7 cM for a total length of 1330 cM (Laurent et al., 1998). For *Nasonia*, a RAPD marker-based linkage map has been developed using the hybrid progeny of a cross between *N. vitripennis* and *N . giraulti* (Gadau et al., 1999). Ninety-one markers covered a total genome length of 764.5 cM with an average space between the markers of 8.4 cM. Pannebakker et al. (2004a) described the linkage map of *Leptopilina clavipes*. Using AFLP markers, five linkage groups were found spanning a total distance of 219.9 cM. More recently, high-throughput techniques have facilitated the production of many maps with far higher numbers of markers, at higher resolution. Niehuis et al. (2010) used the discovered SNP polymorphism between two parental lines to generate a SNP chip and map of 1255 markers (average distance between markers = 0.3 cM) which

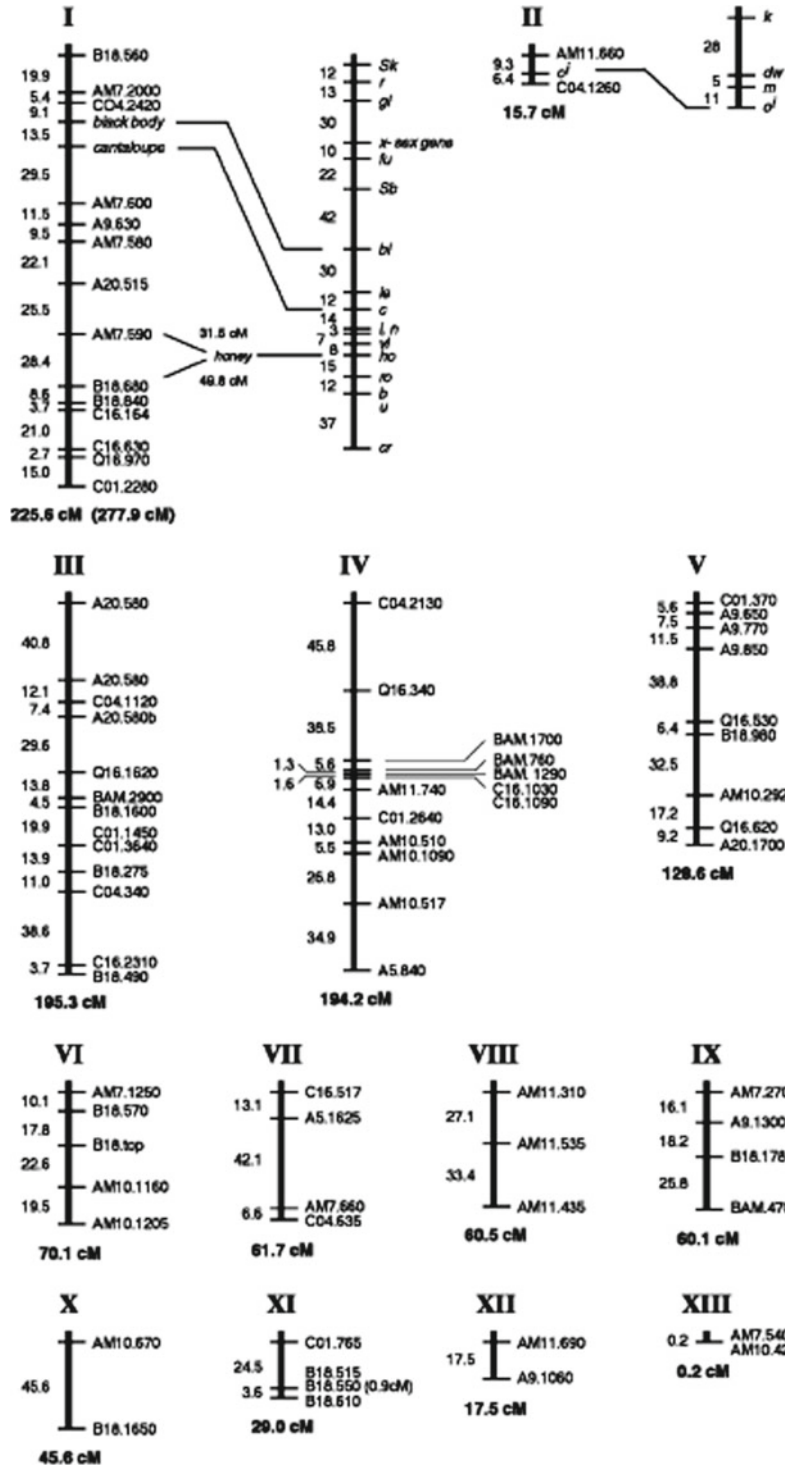


Fig. 3.8 Linkage map of *Habrobracon hebetor*. There are thirteen linkage groups (chromosomes). Groups I and II show the relationship between the maps based on molecular markers (Antolin et al., 1996) and morphological markers (Whiting, 1961). Markers are shown on the right of each group and designated with the primer name and fragment size in bp of DNA. Distances between markers (cM) are shown at the left of each group, and total linkage group length appears at the bottom of each group. *Source* Antolin et al. (1996). Reproduced by permission of the Genetical Society of America

helped in the assembly of the physical map and allowed scaffold orientation. T. M. O. Majerus (unpublished) used RAD-Seq to generate linkage maps with around 15,000 sequenced markers, for each of the 2-spot and 10-spot ladybirds (*Adalia bipunctata* and *Adalia decempunctata*).

Nature and Number of Markers

The availability of PCR and NGS sequencing-based techniques make generation of linkage maps with thousands of markers relatively straightforward. In addition, ambitious projects such as the Earth BioGenome Project, which aims to sequence and characterise the genomes of all eukaryotes (Lewin et al., 2018), may generate reference resources for thousands of insect natural enemies. The remaining challenge perhaps being to identify, and (if necessary) breed, appropriate representative individuals to facilitate investigation of the features of interest.

To obtain a general idea of how many markers (n) are needed to span a certain size of genome (L, cM) the following formula can be used (Lange & Boehnke, 1982):

$$n = \ln(1-p)/\ln(1-2m/L) \qquad (3.4)$$

with p the fraction of loci within m map unit of some marker. This equation assumes a circular genome and corrections can be made for discrete chromosomes (Bishop et al., 1983), thus incorporating chromosome ends. Ignoring chromosome ends tends to underestimate n (Lynch & Walsh, 1998). If, for instance, one wanted to know how many (additional) random markers would be needed to get the spacing for the *Trichogramma* map down to 10 cM, Eq. (3.4) could be used as an approximation. For $p = 0.9$ (i.e., the probability that at least one marker is within 10 cM of a randomly chosen gene is 90%), the total number of markers would be 152. Therefore, 68 additional markers will have to be developed.

Where a sequenced genome is available, individual markers can be anchored to the physical sequence of the genome. This means that by comparison of genetic and physical distances for markers that map to the same sequence contig, it is possible to estimate the relationship between physical and mapping distances, in other words how many cM per Mb. For most species, there is some variation of recombination rates across the chromosome, which make cM/Mb dependent on position on the chromosome.

3.3 Genetics of Reproductive Mode

3.3.1 Introduction to the Genetics of Reproduction

This section discusses some of the genetic consequences of sexual and parthenogenetic reproduction found among insect natural enemies. It focuses mainly on parasitoid wasps because they are one of the most extensively studied groups of insect natural enemies.

3.3.2 Arrhenotoky

As mentioned in the introduction, Hymenoptera and Thysanoptera and some other insects are haplodiploid. The most common mode of haplodiploid reproduction is arrhenotoky, in which male progeny develop parthenogenetically from unfertilised (haploid) eggs and female progeny from fertilised (diploid) eggs. As a result, sons receive genetic material from their mother only, and are therefore 100% related to their mother and unrelated to their father. On the other hand, daughters receive one haploid copy of their genome from each of their parents and are therefore 50% related to their mother and 50% to their father (Fig. 3.9). Unmated females can lay only haploid eggs and so produce progeny consisting solely of males. Mated females store sperm in a spermatheca and can control the sex of their offspring when ovipositing, by selectively releasing sperm to an egg as the latter passes down the common oviduct (Godfray, 1994)

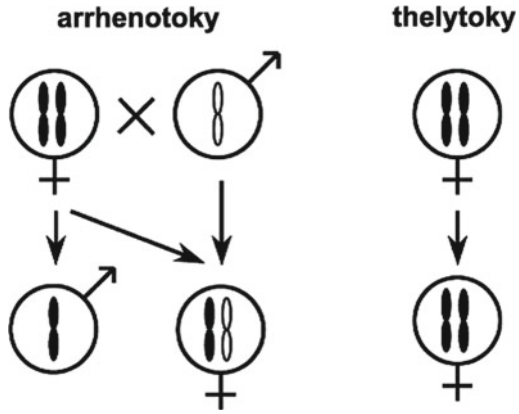


Fig. 3.9 Modes of haplodiploid reproduction. In arrhenotoky males develop parthenogenetically from haploid unfertilised eggs and females from diploid fertilised eggs. In thelytoky females develop parthenogenetically from unfertilised diploid eggs

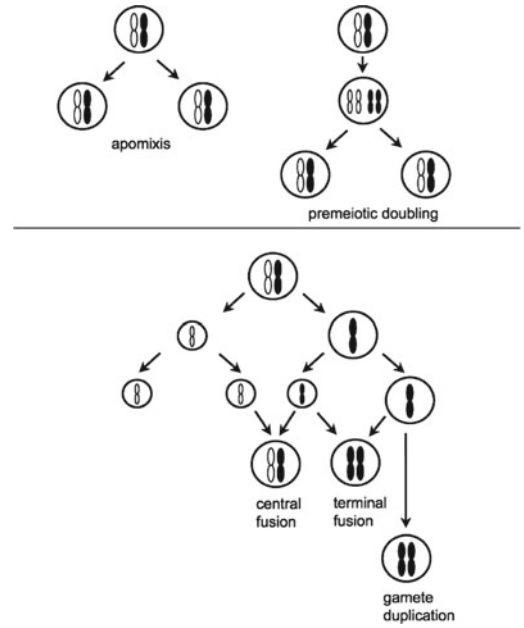


Fig. 3.10 Several cytological modes of parthenogenesis. Apomixis is mitotic parthenogenesis. Premeiotic doubling consists of a duplication of all chromosomes before meiosis followed by a reduction division. Central fusion refers to the fusion of two second polar nuclei and central fusion to fusion of the egg nucleus with the second polar body. Gamete duplication is the doubling of chromosomes without cell division after meiosis. Apomixis and premeiotic doubling result in offspring identical to the mother. Gamete duplication leads to complete homozygosity in one generation. Under terminal and central fusion heterozygosity may be maintained but will decrease through crossing-over

3.3.3 Thelytoky

A minority of species reproduce entirely parthenogenetically by thelytoky. With thelytoky there are no males, and unfertilised eggs give rise to diploid females, resulting in 100% relatedness of daughters to their mother (Fig. 3.9). Thelytokous reproduction occurs sporadically in almost all major parasitoid families (Crozier, 1975; Luck et al., 1993; Quicke, 1997). Schneider et al. (2002) investigate the parasitoid *Venturia canescens* which has both thelytokous and arrhenotokous populations. Thelytokous reproduction can be recognised by initiating isofemale lines from field-collected individuals and scoring the sex of their progeny. Arrhenotokous females will produce sons and daughters when mated or exclusively sons when unmated, whereas thelytokous females will produce only daughters. It is highly advisable to screen a large number of offspring, because sex ratios in a particular species may be strongly female-biased for reasons other than thelytoky (Chaps. 1, 5 and 6) and males may be missed (see below).

There are a number of cytological mechanisms by which thelytoky occurs and these have different effects on the genetic variation within and among offspring (Fig. 3.10). Knowledge of these mechanisms is important for understanding to what extent genetic variation can be maintained in asexual species. A first indication of the actual mechanism may be obtained from parent–offspring analyses with molecular markers. Establishment of the exact mechanism will require careful cytological investigations. Depending on the timing of the process, i.e., pre-, peri- or postmeiotic, developing oöcytes in ovaries, or eggs that have recently been oviposited, need to be examined. Eggs are typically arrested in meiosis stage I during ovigenesis and resume development after oviposition (Went, 1982). The following are general descriptions of the most prevalent modes of thelytokous reproduction. However, note that several modifications and aberrations of these processes have been described.

Apomixis

Apomictic parthenogenesis involves mitotic oögenesis, i.e., eggs are produced by a single mitotic division and there is no reduction division (Fig. 3.10). This mode of reproduction is relatively rare in insects, although it has been reported from several insect orders, including Orthoptera, Homoptera, Diptera and Hymenoptera, (Suomalainen et al., 1987), including from the parasitoid wasp *Meteorus pulchricornis* (Tsutsui et al., 2014). Apomictically produced offspring are identical to their mother.

Automixis

Parthenogenesis by automixis is more common than by apomixis and occurs through meiotic oögenesis, i.e., a reduction division in combination with a process of chromosome doubling known as diploidy restoration. Diploidy restoration can occur in a number of ways which have different consequences for the genetic variation among offspring.

Premeiotic Doubling

Premeiotic doubling is an automictic mechanism in which chromosome doubling occurs before meiosis and the first meiotic division restores the diploid state (Fig. 3.10). If crossing-over occurs, it has no effect because identical sister chromosomes pair. Consequently, offspring are identical to their mother and all heterozygosity is maintained as in apomixis. There are no examples from insects that are natural enemies of other insects (Suomalainen et al., 1987).

Terminal Fusion

In meiosis there are two divisions of the oögonium (Fig. 3.10). The first (meiosis I) is reductional and results in two haploid daughter cells, the egg cell and the first polar body. Both cells divide once more mitotically (meiosis II) resulting in two haploid daughter cells each. The daughter cells of the first polar body degenerate. The egg cell yields the egg and the second polar body. Whereas in the normal case this second polar body also degenerates, under terminal fusion it fuses with the egg cell yielding a diploid egg. Because these two cells arose from a single haploid egg cell in meiosis stage I in which crossing-over may have taken place, terminal fusion leads to increasing homozygosity of loci proximal of cross-overs (i.e., between centromere and cross-over). This type of thelytoky has been reported from the chalcid wasp *Aphytis mytilaspidis* (Rössler & DeBach, 1973).

Central Fusion

With central fusion, one of the two daughter cells of the first polar body does not degenerate but fuses with the second polar body giving rise to the diploid egg. The egg cell and the other second polar body degenerate. Although this process can retain heterozygosity, it leads to an increase of homozygosity each generation. Whenever a cross-over occurs between both homologues in the oögonium, it can result in identical copies in the egg, depending on the assortment. Loci close to the centromere have a lower cross-over rate and therefore a higher chance to be retained in a heterozygous state. Central fusion is known from the ichneumonid wasp *Venturia canescens* (Speicher et al., 1965; Beukeboom & Pijnacker, 2000).

Gamete Duplication

Gamete duplication refers to chromosome doubling during the first cleavage division of the mature egg. It basically involves endomitosis, i.e., chromosome replication without a following cell division. All cases of *Wolbachia*-induced thelytoky (see Causes of Thelytoky below) involve gamete duplication. It has been found in several parasitoid wasps including *Trichogramma*, *Muscidifurax* and *Encarsia* (Stouthamer, 1997) and the *Drosophila* parasitoid *Leptopilina clavipes* (Pannebakker et al., 2004b). Because all chromosomes are simply doubled, this mechanism results in complete homozygosity within one generation.

Causes of Thelytoky

Although thelytoky is generally considered to be derived from arrhenotoky, the genetic factors that induce thelytoky are almost completely unknown. One cause of thelytoky is interspecific

hybridisation (Nagarkatti, 1970; Pintureau & Babault, 1981; Legner, 1987). Nagarkatti and Fazaluddin (1973) crossed a female of *Trichogramma perkinsii* with a male of *T. californicum*, and the offspring comprised one thelytokous female, seven arrhenotokous females and ten males. Tardieux and Rabasse (1988) observed that females of *Aphidius colemani* produced daughter offspring thelytokously when mated to males of a closely related species, whereas only males were produced if courtship was interrupted and copulation prevented. Arrhenotokous reproduction was excluded because all daughters had the electrophoretic esterase pattern of their mother.

Another cause of thelytoky is the presence of microbes (Stouthamer et al., 1990, 2002). In several parasitoid wasp species thelytoky is caused by intracellular *Wolbachia* bacteria (Sect. 6.5). Stouthamer et al. (1990) showed that if such species are treated with antibiotics or exposed to high temperatures, which will kill the bacteria, they will produce both sons and daughters. Pijls et al. (1996) showed, by treating the thelytokous wasps with antibiotics, that the arrhenotokous population of *Apoanagyrus* (=*Epidinocarsis*) *diversicornis* attacking the cassava mealybug, *Phenacoccus manihoti*, in Central South America, is conspecific with morphologically identical but thelytokous populations in northern South America that attack *P. herreni*. Males were obtained that interbred successfully with females of the arrhenotokous population. However, this reversion to arrhenotokous reproduction is not always successful because males are frequently non-functional (see below). One final example of the use of heat treatment to reverse what was perhaps the impact of endosymbionts, is the extraordinary case of *Micromalthus debilis*. This species of beetle reproduces by parthenogenetic paedogenesis, i.e., without the production of adults. Perotti et al. (2016) were able to artificially induce adult beetles to investigate its pre-paedogenetic mating system.

Both arrhenotokous and thelytokous forms are known in several parasitoid wasp species and these forms occur either allopatrically or sympatrically (Stouthamer, 1993). In several such species *Wolbachia* bacteria are absent and thelytoky must be caused by another mechanism. Although other parthenogenesis-inducing microorganisms might exist, it is likely that parthenogenetic egg production in such species has a genetic basis. In addition, genetic variation for parthenogenesis may be present in arrhenotokous populations, but this remains to be investigated. In *Spalangia endius*, Bandara and Walter (1993) found that virgin arrhenotokous females occasionally produced daughters from unfertilised eggs, but these daughters in turn reproduced arrhenotokously. This phenomenon was originally reported from *Habrobracon hebetor* by Speicher (1934). Similarly, Beukeboom et al. (1999) found daughter production from unfertilised eggs of arrhenotokous *Venturia canescens* females at a frequency of approximately 0.2%, but this ability was not transferred to the next-generation. Thus, thelytokous reproduction seems to occur sporadically in arrhenotokous species, but whether this can lead to stable thelytokous lineages remains to be established.

The extent to which thelytokous species can be considered as purely clonal is questionable. In general, individuals are either sexual or obligately asexual. However, in *Trichogramma* and *Aphytis*, thelytokous females were found to mate with sexual conspecific males and use their sperm to fertilise their eggs (Rössler & DeBach, 1973; Stouthamer & Kazmer, 1994). These examples concern *Wolbachia*-induced thelytoky and post-meiotic gamete duplication, which suggests that such forms of thelytoky are genetically isolated to a lesser degree than pre- and peri-meiotic forms. Besides direct observation, evidence for gene flow between sexuals and asexuals may be obtained from genetic marker studies. Mitochondrial DNA, which is only maternally inherited, may be informative about the frequency at which asexuals arise from sexuals.

3.3.4 Deuterotoky

Deuterotoky refers to female production with rare males. It differs from arrhenotoky in that both sexes develop from unfertilised eggs

Table 3.1 Reproductive modes in haplodiploid insects

Reproductive mode	Males from	Females from	Type
Arrhenotoky	Unfertilised eggs	Fertilised eggs	Sexual
Thelytoky	Absent	Unfertilised eggs	Asexual
Deuterotoky	Unfertilised eggs	Unfertilised eggs	Asexual

(Table 3.1). However, as pointed out by Luck et al. (1993), the distinction between thelytoky and deuterotoky is ambiguous, the reason being that some parasitoid wasp species originally designated as thelytokous have been found to produce small numbers of males. These males are produced when the maternal females have been exposed to high temperatures. Whilst they have been considered to be non-functional, there is evidence that in some cases they are not only capable of mating but also able to pass on their genes to progeny, which reproduce by thelytoky. Some groups such as the gall-causing herbivorous Cynipidae alternate between two reproductive modes, i.e., deuterotoky and arrhenotoky or thelytoky. As already mentioned, the underlying genetics of these different modes of reproduction remains a challenging field.

3.4 Genetics of Sex Determination and Sex Ratio

3.4.1 Genetics of Sex Determination

Sex Chromosomes

A variety of sex-determining mechanisms is known from insects and includes male and female heterogamety, haplodiploidy, paternal genome loss and systems with X chromosome elimination (Metz, 1938; Hughes-Schrader, 1948; Crozier, 1971; Bull, 1983; Nur, 1989; Cook, 2002). The most widespread mechanism is heterogamety, in which one sex has two identical sex chromosomes (= homogametic sex, e.g., XX) and the other two different ones (= heterogametic sex, e.g., XY). The heterogametic sex is more often the male (XY) than the female (ZW). Female heterogamety is indicated with ZZ/ZW for distinction. Loss of the Y chromosome has resulted in an XO system in several insect groups such as for example praying mantids (White, 1954).

Heteromorphic sex chromosomes can easily be detected cytologically. Sex chromosomes are often morphologically different from the autosomes, i.e., they contain more heterochromatin, condense out of phase or are of different size. However, there are cases in which the sex-determining genes are present on the autosomes and it is impossible to distinguish between sex chromosomes cytologically. For example, in the non-parasitoid *Megaselia scalaris* (Phoridae, some members of which are parasitoids), a single gene determines maleness and varies in its position in the genome (Mainx, 1964). Traut (1994) used phenotypic and molecular markers to map this gene to various chromosomes in different stocks. Blackmon et al. (2017) provide an excellent overview and have produced a database of karyotypes, sex chromosomes and sex determination for over 13,000 species, covering 29 orders of insects.

Haplodiploidy

All Hymenoptera are haplodiploid; there are no heteromorphic sex chromosomes. Sex is determined by the number of chromosome sets present in the embryo. We first discuss general methods of assessing chromosome number and then the application of these to identifying the chromosome composition, and thus sex, of offspring. We then discuss aspects of sex determination among haplodiploids that go beyond ploidy itself.

Establishing Chromosome Numbers

A general introduction to animal cytogenetics is given by MacGregor (1993). An important number of works have been published on

parasitoids (e.g., Gokhman, 2009, 2021; Gokhman et al., 2016, 2017). Such investigations facilitate understanding of taxonomy (Gokhman, 2006) and are most effective at species level, for example in the identification of species where distinction based on phenotype is difficult or impossible (two species that look identical but are actually cryptically separate species), or where identification is only possible in adults. They also provide invaluable insights into understanding the primary sex ratio (see below).

The most suitable tissues for establishing chromosome numbers (karyotypes) are male and female gonads, but brain tissue of larvae can also be used (e.g., Traut & Willhoeft, 1990). It should be noted that other somatic tissues, such as muscles, frequently undergo endopolyploidisation (Aron et al., 2005), making chromosome counts both very difficult and unreliable. The number of metaphase plates (a phase of maximal contraction of chromosomes during cell division) may be increased by feeding the animals colchicine (0.15%) for a few hours (longer periods can lead to polyploidisation of cells). One can use freshly eclosed adult males, but some authors have found the gonads of male larvae or pupae to yield more division figures. Females are typically allowed to lay eggs for a while before dissection of ovaries in order to ensure that they are in an active egg-laying condition.

Animals can be dissected in either water or Ringer's solution and the gonads immediately transferred to Carnoy's fixative (3 parts methanol or ethanol: 1 part glacial acetic acid, optionally one can add 2 parts chloroform). Gonads are then transferred to a drop of 45–70% acetic acid on an object glass, torn into fine pieces and then squashed. Several stains can be applied including: (1) 2.5% lacmoid (2.5% lacmoid in 1 part water: 1 part lactic acid: 1 part acetic acid); (2) 2% Giemsa (in Sörensen buffer: 55 ml 1/15 M Na_2HPO_4 plus 1/15 M KH_2PO_4, pH 6,9); (3) Feulgen (=Schiff's) reagent: dissolve 1 g pararosaniline in 30 ml 1N HCl and 1 g $K_2S_2O_5$ in 170 ml demiwater, combine both solutions and destain for 24 h in the refrigerator, shake solution with 600 mg Norit for 2 min and filter through Whatman paper, store in a tight bottle in refrigerator, solution must always smell of SO_2, if not add tiny amount of $K_2S_2O_5$; (4) carmine: add 4 g carmine and 1 ml concentrated HCl to 15 ml distilled water, boil and stir for 10 min, cool and add 95 ml of 85% alcohol, and filter, Snow, (1963); or (5) fluorescent 4′,6-diamidine-2-phenylindole-dihydrochloride (DAPI). Slides can be temporarily prevented from drying out by sealing them with nail polish. A more permanent method is to freeze them on dry ice, snap off the cover slip with a razor blade, air dry and seal them with DePeX (Sumner, 1972).

Assessing the Sex of Haplodiploid Immatures

Cytological techniques for counting chromosomes of haplodiploid parasitoids can be used to establish the sex of freshly laid eggs (Dijkstra, 1986; van Dijken, 1991). For instance, van Dijken (1991) described the method for *Apoanagyrus lopezi*: eggs are dissected from hosts and placed in a droplet of 2% lacto acetic orcein (a chromosome stain made by dissolving 1.0 g of natural orceine in 10 ml of 85% lactic acid, 25 ml of glacial acetic acid and 15 ml of distilled water; this mixture is gently boiled for 1 h then cooled and filtered) and a cover slip gently placed over them. The eggs are then squashed to a mono-layer after 25 min have elapsed and the cover slip sealed with nail varnish. After staining for 24 h at room temperature the chromosomes are examined and counted. Exact counts are unnecessary, as females have two sets of chromosomes which is easily distinguished from one set in males (Fig. 3.11). The optimum time to fix and squash the eggs may vary between species (e.g., 18–24 h in *Apoanagyrus lopezi*), so it is advisable to make a series of egg squashes at different times following oviposition. In haplodiploids, unfertilised eggs of virgin females can be used as control to determine the haploid number of chromosomes (see also Ueno & Tanaka, 1997).

More recently the sex of eggs laid by the gregarious parasitoid *Goniozus legneri* has been identified using DNA microsatellite markers (Sect. 3.2.2, see also Liu et al., 2023). The

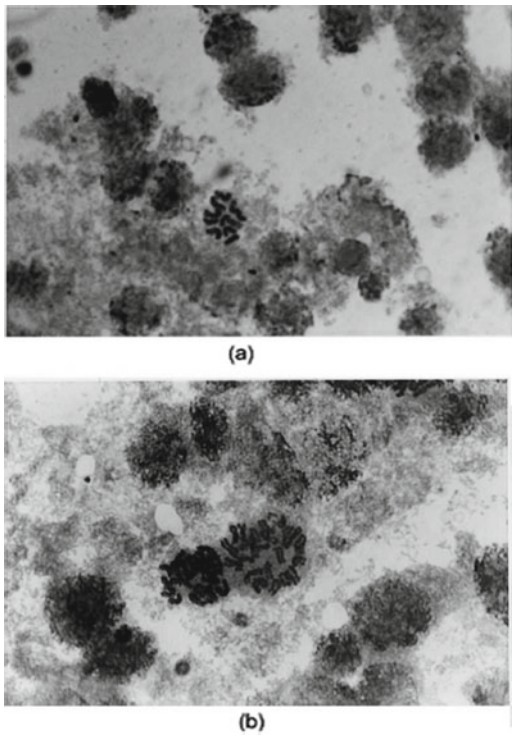


Fig. 3.11 Metaphase chromosomes in eggs of *Apoanagyrus lopezi*: (**a**) haploid male and (**b**) diploid female. See text for methodology. Reproduced by kind permission of M. J. van Dijken

technique involves finding between-strain polymorphism, such that a strain carrying only one allele can be crossed with a strain carrying a different allele and thus eggs in which only one allele is detected (hemizygous) are identified as male and those containing both alleles (heterozygous diploids) are female (Khidr et al., 2013, 2014). Note that while diploid males occur in some Hymenoptera, due to complementary sex determination mechanisms (see below), these are extremely unlikely to operate in *Goniozus* (Cook, 1993a).

Whichever method they are obtained by, data on the sex of parasitoid eggs can be used to circumvent or assess the effects of developmental mortality that can operate to affect or obscure sex allocation strategies in parasitoids (Hardy et al., 1998; Khidr et al., 2013; Wilkinson et al., 2016; Liu et al., 2023; Chap. 2).

Single-Locus Complementary Sex Determination (slCSD)

Under single-locus complementary sex determination (slCSD), sex is determined by multiple alleles at a single locus. Heterozygosity leads to female development, but hemizygotes and homozygotes develop into males. This mode of sex determination was first described by Whiting (1939, 1940, 1943) from *Habrobracon hebetor*. It has been demonstrated in all major suborders of the Hymenoptera (reviewed in Luck et al., 1993; Periquet et al., 1993; Cook, 1993b, 1993c, 2002; Butcher et al., 2000; van Wilgenburg et al., 2006) but appears to be absent from the superfamily Chalcidoidea. The estimated number of alleles at the sex locus typically range from 10 to 20 (Cook & Crozier, 1995) but can be as high as 86 in fire ants (Ross et al., 1993; see also Adams et al., 1977, for methods of estimating the number of alleles).

Single-locus CSD can easily be detected with inbreeding studies. The most straightforward method is mother–son mating. This is possible if females first reproduce as virgins and can be kept alive long enough to be mated with one of their sons. The resulting progeny will only carry two sex alleles and half of the fertilised (diploid) eggs will be homozygous for the sex allele and develop into diploid males (Fig. 3.12a). Note that there will also be males developing from unfertilised (haploid) eggs. If mother–son crosses are not possible, son-daughter (i.e. brother–sister) matings can be used. Such crosses can be matched (two-allelic) or unmatched (three-allelic) depending on whether the son inherited the same or a different allele as the daughter (Fig. 3.12b). If both carry the same allele, the diploid offspring will be 50% homozygous and male (similar to mother–son crosses), but if they carry different alleles 100% of diploid eggs will be heterozygous and female. As 50% of brother–sister matings will, by chance, be matched and 50% will be unmatched, on average 25% of fertilised eggs are expected to become diploid males in brother–sister crosses. This will be manifested in an increase in the sex ratio of

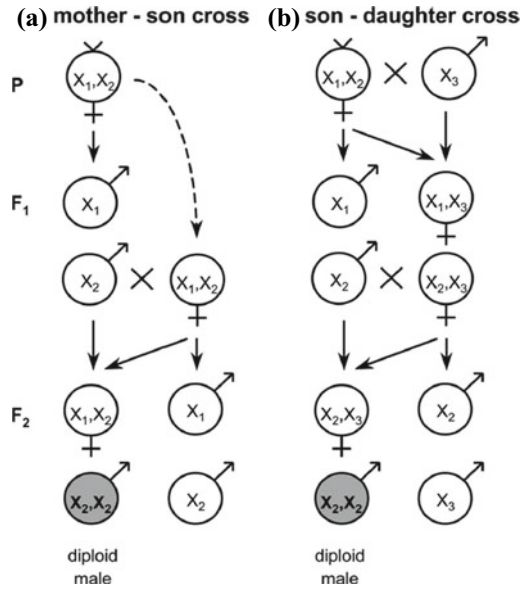


Fig. 3.12 Inbreeding crosses to detect single-locus complementary sex determination. (**a**) Mother–son crosses. Diploid heterozygous females produce two types of hemizygous sons. In back-crosses with their mother both types yield 50% diploid homozygous sons among fertilised eggs. (**b**) Son–daughter crosses. Diploid heterozygous females are mated with their hemizygous brothers. Half of these crosses are matched (two-allelic) and result in 50% diploid homozygous sons among fertilised eggs, whereas the other half are unmatched (three-allelic, not shown) and do not result in diploid males. On average, 25% of fertilised eggs are expected to become diploid males in brother–sister crosses

inbred crosses. However, caution should be exercised because diploid males may be inviable. For example, in *Habrobracon hebetor*, diploid male embryos frequently die, but hatchability of such eggs can be restored under mineral oil (Petters & Mettus, 1980). It is therefore important to also compare brood sizes and the number of non-emerged hosts in inbred and control (non-inbred) crosses (Beukeboom, 2001).

Diploid males can be recognised in a number of ways. Genetic identification is the most reliable method, i.e., through the use of genetic markers or cytology. Morphological identification is also possible but not always conclusive. Diploid males are typically larger than haploid males, and because they have larger cells, their bristle spacing on the wings is larger (e.g., Grosch, 1945). Although diploid males have been found in approximately 50 species of Hymenoptera, they are not always the result of homozygosity at the sex locus but, instead, may have arisen by mutation.

Species with a slCSD mode of sex determination are difficult to maintain in culture and are often lost due to a diminishing number of sex alleles and a concomitant increase in (sterile) diploid males (Stouthamer et al., 1992; Cook, 1993c). Problems with culturing may, therefore, provide a first indication for this mode of sex determination. It also means that one should be cautious if using parasitoids that have been in culture for some time, when testing for slCSD. Cook (1993a) provided a quick test based on brood survival and secondary sex ratio to determine whether one is dealing with an inbred population that suffers from mortality of diploid males due to a single-locus two-allele system of sex determination (Fig. 3.13). If 50% of fertilised eggs are male and fertilisation proportion is 100%, the primary sex ratio will be 50% (diploid) males in the absence of diploid male mortality, indicated by the right side of the graph. The left side of the graph corresponds to a situation in which all diploid males would die if fertilisation were 100%, resulting in a sex ratio of 0, and 50% of eggs developing into (surviving) females. Thus, if values fall outside the

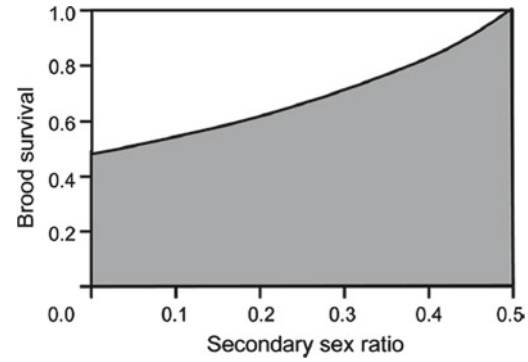


Fig. 3.13 Test for single-locus sex determination using brood survival and secondary sex ratio. Strains falling outside the shaded area cannot have single-locus CSD. Points falling in the shaded area indicate that the experimental strains may have CSD. *Source* Cook (1993a). Reproduced by permission of Blackwell Publishing

shaded area, there is insufficient mortality to generate the degree of female bias observed and slCSD is not possible. There are, however, a number of limitations to the test: (1) it only applies to species with female-biased sex ratios and measurable mortality; (2) it cannot establish the presence of diploid males; and (3) there may be reasons for reduced brood survival other than diploid male production, e.g., suboptimal hosts or host immune reactions. If this test is not possible or proves inconclusive, other methods need to be used such as those described above.

Alternative Models for Sex Determination

Single-locus CSD is clearly absent in a number of wasp species. Prolonged inbreeding experiments over many generations with the pteromalid *Nasonia vitripennis* (Skinner & Werren, 1980) and the bethylid *Goniozus nephantidis* (Cook, 1993a) did not yield diploid males. Several alternative models have been proposed to explain sex determination in those species, including multi-locus CSD (mlCSD), maternal effect and genomic imprinting (reviewed in Cook, 1993b; Beukeboom, 1995). Dobson and Tanouye (1998) presented some evidence for a role of genomic imprinting in sex determination in *N. vitripennis*, but there are alternative interpretations of their results (Beukeboom et al., 2000). More recently, Cook et al. (2018) have investigated whether the control of sex determination in *N. vitripennis* is based on methylation-based imprinting, without an obvious change in sex allocation after changing methylation patterns using 5-azacytidine and bisulfite sequencing. However, they did note that both decreases in methylation (as expected for the non-methylatable 5-azacytidine) and increases in methylation were observed throughout the genome.

A slCSD or mlCSD mechanism of sex determination is likely to be absent in some species because it conflicts with their population biology or reproductive mechanism. Selection against CSD is expected with a population structure that is characterised by high inbreeding. This would impose a strong genetic load on a population because diploid males are typically sterile and less viable (Crozier, 1977; Cook & Crozier, 1995). Many parasitoid wasp species, including several chalcidoids, exhibit strong natural inbreeding (Godfray, 1994; Hardy, 1994; West, 2009; Chaps. 4 and 5). In addition, certain forms of thelytokous reproduction lead to complete homozygosity (see above). This is inconsistent with CSD because all such offspring should develop into diploid males rather than females (reviewed by Cook, 1993b).

Gynandromorphs and Intersexes

A gynandromorph or sex mosaic is an individual which has both male and female characteristics. Such individuals have been reported from many insect orders. Some examples are shown in Fig. 3.14. Gynandromorphs can arise in a number of ways. In diploid organisms, gynandromorphs usually result from loss of one sex chromosome in some cell lineages. In haplodiploids, gynandromorphs are often mosaic for haploid and diploid tissue. They can arise from mitotic loss of one set of chromosomes in certain cell lineages, or from fertilisation of one nucleus in a binucleate egg (post-cleavage fertilisation). Cold treatment of newly laid eggs can induce gynandromorphism in *Habrobracon* (Greb, 1933; Petters & Grosch, 1976). The behaviour of gynandromorphs is determined by the sex of the brain (Whiting, 1961; Clark & Egen, 1975).

Intersexes are individuals intermediate between a normal male and female (Goldschmidt, 1915). They are genetically uniform individuals and differ from gynandromorphs, which have mixtures of male and female parts. They arise from a disturbed balance of the expression of female- and male-determining genes.

3.4.2 Genetics of Sex Ratio

Quantitative Genetics of Sex Ratio

Sex ratio is usually expressed as the proportion of males among progeny (however, this is merely a convention and some authors have expressed it as the proportion of females, e.g., Fig. 3.15). Most diploid organisms have equal proportions

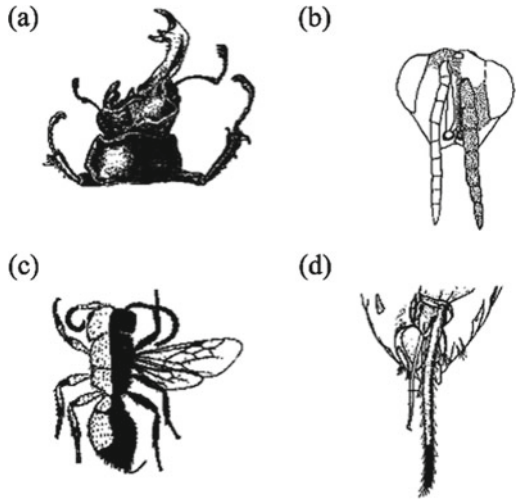


Fig. 3.14 Examples of gynandromorphic animals. (**a**) Head and thorax of the stag beetle *Lucanus cervus*, right = male (head with sculptured ridges, large mandible, long antenna); left = female (head without ridges, small mandible, short antenna). *Source* Stern (1968) after Dudich (1923). Reproduced by permission of Gustav Fisher Verlag GmbH & Co.. (**b**) Frontal view of the head of the chalcid *Hockeria rubra*, right (stippled) = male and non-stippled is female. *Source* Halstead (1988). Reproduced by permission of the Entomological Society of Washington. (**c**) Habitus of solitary wasp *Pseudomethoca canadensis*, right = male (thirteen antennal segments, two ocelli, wings, male-type legs) and left = female (twelve antennal segments, no ocelli, no wings, female-type legs). *Source* Stern (1968) after Wheeler (1910). Reproduced by permission of the Cambridge Entomological Club. (**d**) Ventral view of genitalia of *Habrobracon hebetor*, a complete set of male genitalia is present as well as a female ovipositor. *Source* Stern (1968) after Whiting (1940). Reproduced by permission of *The Biological Bulletin*

of males and females (sex ratio = proportion males = 0.5). In contrast, haplodiploid organisms frequently have female-biased sex ratios (sex ratio < 0.5). The primary sex ratio refers to the initial proportion of male eggs immediately after oviposition. It can be determined using cytological techniques or through molecular assessment (Sect. 3.4.1). Due to differential mortality of the sexes during egg, larval or pupal development, the secondary sex ratio may be very different from the primary sex ratio (Khidr et al., 2013; Wilkinson et al., 2016).

Although there have been numerous theoretical and empirical studies of sex allocation decisions in the Hymenoptera (e.g., Charnov, 1982; Hardy, 1992, 1994, Wrensch & Ebbert, 1993; Antolin, 1993; Godfray, 1994; 2002; Ode & Hunter, 2002; West, 2009; Gardner & Hardy, 2020; Abe et al., 2021; Iritani et al., 2021; Guo et al., 2022; Lehtonen et al., 2023), the genetic basis of sex ratios is much less studied. There are several approaches for studying the genetic basis of sex ratios: (1) breeding of iso-female lines, (2) selection experiments, and (3) quantitative genetic analyses. A first indication for the existence of genetic variation can be obtained from scoring progenies of field-collected iso-female lines under similar laboratory conditions. Such geographical variations in sex ratio have been reported for a number of species (e.g., *Muscidifurax raptor* (Antolin, 1992) and *Nasonia vitripennis* (Orzack & Parker, 1986, 1990; Orzack et al., 1991), suggesting the existence of autosomal genetic variation for sex ratio.

The second approach is to attempt to select for sex ratio. Orzack and Parker (1986) were able to produce high (45% male) from low (10% male) sex ratio lines in *N. vitripennis* by selectively breeding over fifteen generations those females which produced the highest proportion of males each generation (Fig. 3.15). Similarly, Wilkes (1964) was able to select the sex ratio from the normal 8% to 95% males in *Dahlbominus fuliginosus*. In fact, in such experiments one selects for the proportion of eggs that are fertilised which may have very different underlying causes. Selection in *N. vitripennis* apparently involved major sex ratio genes as well as genes for behaviour of females (Parker & Orzack, 1985). In contrast, the results in *D. fuliginosus* were due to reduced functionality of sperm and had a physiological basis (Wilkes & Lee, 1965).

The third approach to the study of genetics of sex ratio is to use quantitative genetic techniques such as parent–offspring regressions to determine narrow and broad sense heritabilities (Sect. 3.5). There are two phenomena that need to be considered in studies of the genetic basis of sex ratio, because they may lead to false conclusions. First,

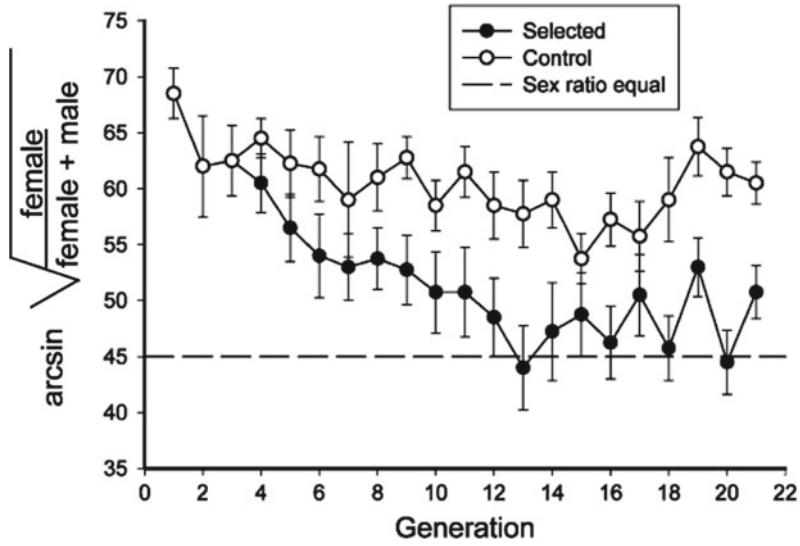


Fig. 3.15 Response to selection on sex ratio in five lineages of *Nasonia vitripennis*. Data are expressed as the arcsin (in degrees) of the square root of the proportion females. The vertical bars indicate 95% confidence limits for the broods in each line and generation; selected lines (solid circles) and control lines (open circles). The horizontal dotted line represents equal (one-to-one) sex ratio. Individual selection for an increased proportion of males was performed on two replicate lines established from a heterozygous stock of wasps produced by two generations of crosses among wasps from five localities. From each generation, the ten broods in each line with the lowest proportion of females were chosen to contribute the parents for the next-generation. In the control line, selected lines were chosen at random with respect to sex-ratio (Parker & Orzack, 1985). Redrawn from Fig. 2B of Parker & Orzack (1985). Reproduced by permission of The Genetical Society of America

as Luck et al. (1993) and Stouthamer et al. (1992) point out, one should be cautious with selection experiments for sex ratio in species with a CSD mechanism of sex determination. In such species, the number of sex alleles may rapidly decrease, leading to a highly male-biased sex ratio. Clearly, in such cases, an increase in sex ratio is the result of the underlying sex-determining mechanism rather than a response to selection on genes involved in the process of egg fertilisation. The second phenomenon is the widespread existence of nuclear and cytoplasmic sex ratio distorters that may overrule the plasticity in sex ratio response of individual females (see below).

Finally, using parent–offspring regression, Wajnberg (1993) demonstrated significant intra-population genetic variation in the order in which son and daughter eggs are laid by *Trichogramma brassicae* females. This was shown to modify the sex ratio produced by the mothers, that is thus also genetically variable (Wajnberg, 1994).

More research on the genetic basis of sex-ratios is needed. Little is known about genetic correlations between sex ratios and other life-history traits (e.g., diapause, Orzack & Parker, 1990). Moreover, many sex ratio models assume that sex ratio is controlled by many genes with small effect (Antolin, 1993), but this assumption largely remains to be tested. With the development of genomic mapping and as sequence data become available, major progress in this field is occurring e.g., identification of gene expression differences after mating in *Anastatus disparis* (Liu et al., 2019) and between male and female *Brachymeria lasus* (Liu & Hao, 2019); investigation of sex ratio determination in *Nasonia vitripennis* via QTL (Pannebakker et al., 2011) and GWAS (Pannebakker et al., 2020), whilst Leung et al. (2020) discuss the need for integration of genetics and genomics to create a framework for 'next-generation biological control'.

Sex Ratio Distorters

Severe forms of sex ratio distortion other than thelytoky and diploid male production are known. The responsible agents vary from extra chromosomes residing in the nucleus to cytoplasmically transmitted organisms including bacteria, viruses and protozoans (Hurst, 1993, Hurst et al., 1996, and Stouthamer et al., 2002, provide overviews of cytoplasmic sex ratio distorters). As a rule they alter the sex ratio towards the sex through which they are transmitted, i.e., most increase the proportion of females because they are inherited through the egg cytoplasm (but see PSR, below). They have therefore been referred to as "selfish genetic elements" leading to "intragenomic conflict" between the autosomes (which are selected to be inherited in a Mendelian fashion) and the sex ratio-distorting element (Werren, 1987; Hurst, 1992). This theory predicts that suppressor genes to sex ratio distortion will evolve on the autosomes. Sex ratio distorters may be much more common than previously thought. There is some evidence from *Drosophila* that sex ratio distorters are frequently kept "in check" by locally adapted suppressor genes but are expressed when crossed into a different genetic background (Merçot et al., 1995; Atlan et al., 1997; Capillon & Atlan, 1999). Much more empirical work on the detection and dynamics of sex ratio distorting and suppressor genes is needed to understand fully their evolution and possible application in biological control (Ode & Hardy, 2008). However, Galizi et al. (2016) describe an exciting and potentially wide-ranging application of CRISPR-Cas9 technology in *Anopheles gambiae* (mosquitoes that are vectors for malaria and dengue fever) which resulted in a strong male-biased sex ratio distortion among the progeny.

Paternal sex ratio (PSR)

The paternal sex ratio (PSR) element is a supernumerary chromosome in the parasitoid wasp *Nasonia vitripennis* that is present in some males (Werren et al., 1981, 1987; Werren, 1991; Stouthamer et al., 2002). Males carrying the element cause females they mate with to produce all-male broods, even though their sperm fertilise the female's eggs. The PSR element destroys the chromosomes which are derived from the sperm nucleus after fertilisation of the egg. The maternal chromosomes are unaffected and because PSR survives itself, the resulting embryo develops into a haploid PSR-bearing male. Thus, PSR converts diploid (female) eggs into haploid PSR (male) eggs. It is considered a "selfish" genetic element because it completely eliminates its host's genes each generation (Nur et al., 1988).

PSR was first discovered while attempting to select for variability in sex ratio control among field-collected strains (Werren et al., 1981). Werren and van den Assem (1986) showed that the trait was strongly correlated with egg fertilisation (Fig. 3.16). Subsequent cytogenetic analyses showed that sperm from PSR-carrying males indeed entered the egg but that one set of chromosomes condensed into a chromatin mass during the first division of the egg (Fig. 3.17) (Werren et al., 1987). Using genetic markers, it was shown that the paternal chromosomes were always destroyed. Cytological investigation of testes from PSR males revealed a small supernumerary (or B) chromosome (Nur et al., 1988) which is absent in control males (Fig. 3.17). PSR males sometimes produced daughters among their offspring; this was shown to be due to occasional failure of the PSR chromosome to be included into sperm of carrier males (Beukeboom & Werren, 1993). Similar elements have been discovered in the parasitoids *Encarsia formosa* (Hunter et al., 1993) and *Trichogramma kaykai* (Werren & Stouthamer, 2003).

Maternal sex ratio (MSR)

Another sex ratio distorter that has been recorded in *Nasonia vitripennis* is the maternal sex ratio (MSR) factor (Skinner, 1982; Stouthamer et al., 2002). MSR females produce broods that consist of daughters only, or rarely contain one or a few males. The nature of the MSR element is unknown, but it is cytoplasmically inherited and probably involves a mitochondrial variant that somehow affects the female's control over the spermatheca.

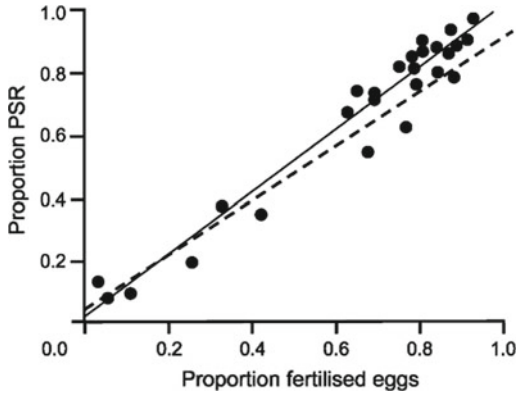


Fig. 3.16 The relationship between the proportion of eggs fertilised by mated females of *Nasonia vitripennis* and the proportion of the male progeny that carry the paternal sex ratio (PSR). The former proportion was determined from control crosses in which male and female wasps were of various PSR-negative genetic strains (the percentage of females in the progeny being the percentage of fertilised eggs, assuming no developmental mortality). The latter proportion was determined for test crosses involving male wasps taken from a known PSR strain and females from the PSR-negative strains. The PSR trait was assayed as follows: females were mated with presumptive PSR males and the sex ratio of the resulting progeny scored. If a greater than 90% male brood resulted, the male parent was taken to be a PSR carrier. This phenotypic test involves a small bias since approximately 5% of control crosses also result in broods containing more than 90% males, as a result of inadequate mating. Where the assay proved ambiguous, at least five males from the F_1-brood were also tested, and if two or more produced all-male broods the grandparent was deemed to be a PSR carrier. The data presented are not corrected for the small bias. There is a strong linear relationship between PSR transmission and egg fertilisation, found both when PSR transmission is assumed to be affected by egg fertilisation (solid line) and when egg fertilisation is assumed to be affected by PSR transmission (dotted line), strongly suggesting that PSR is a factor transmitted from sperm to eggs upon fertilisation. In fact, PSR is an example of a parasitic B-chromosome (B-chromosomes are extra to the normal chromosome complement). PSR destroys the other paternal chromosomes in the early fertilised egg. It disrupts the normal haplo-diploid sex-determination system of *Nasonia* by converting diploid (female) eggs into haploid eggs that develop into PSR-bearing males (Beukeboom & Werren, 1993). Werren and van den Assem (1986) *Source* Cook (1993a). Reproduced by permission of Blackwell Publishing

Male-Killing Microbes

Maternally inherited microbes that kill male but not female hosts during embryogenesis are known from a number of insects, in particular coccinellid beetles and parasitoid wasps (Hurst, 1991). Several widely divergent microbial taxa are involved including *Rickettsia*, *Wolbachia*, *Spiroplasma* and Flavobacteria (Hurst et al., 1996; Stouthamer et al., 2002). The presence of micro-organisms can be readily determined, and their identity established with molecular methods such as PCR amplification and the appropriate primers (Sect. 6.5).

Another bacterium, *Arsenophonus nasoniae*, is the cause of "son-killer" in *N. vitripennis* (Huger et al., 1985; Skinner, 1985; Werren et al., 1986; Gherna et al., 1991). It occurs at low frequencies in natural populations and causes all-female broods by killing male eggs only. The bacterium is present in the female's ovaries and transmitted via the eggs to offspring. It is also injected into the fly pupal host and transmitted horizontally by re-infecting other female larvae in case of superparasitism. Strictly speaking, son-killer affects secondary sex ratios.

In the coccinellid *Adalia bipunctata*, it was noticed that some females produced female-biased sex ratios and that the success of hatching among the eggs laid by such females were low. This was shown to be caused by a *Rickettsia*-like bacterium that kills male embryos (Hurst et al., 1992, 1993). More recent evidence suggests that male-killing bacteria are common in aphidophagous coccinellids. Majerus and Hurst (1997) and Majerus and Majerus (2012) review the known examples, and present and test, respectively, predictions about which species groups are likely to carry male-killers. Majerus and Majerus (2010) describe the identification of a host gene that suppresses male-killing in the ladybird *Cheilomenes sexmaculata*, by rescuing infected males from the pathogenic effects of infection.

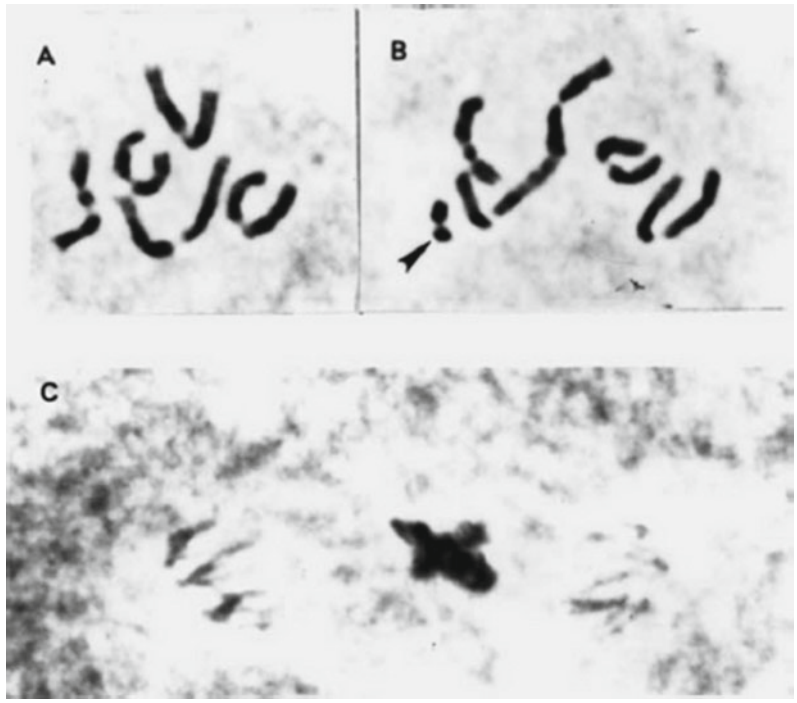


Fig. 3.17 Appearance in spermatogonia, and action in fertilised eggs, of the PSR chromosome in *Nasonia vitripennis*; (**a**) karyotype of a male with five chromosomes lacking PSR; (**b**) karyotype of a male with five chromosomes carrying PSR (arrow). *Source* Nur et al. (1988). Reproduced by permission of the AAAS. (**c**) The appearance of a dense chromatin mass of paternal chromosomes at the second mitotic division in a PSR fertilised egg. Haploid sets of maternal chromosomes are seen in anaphase. The paternal chromosomes do not participate in the mitotic division. *Source* Werren et al. (1987). Reproduced by permission of Macmillan Journals Ltd

Sex ratio-distorting micro-organisms in insects can be detected by exposing them to a high temperature, e.g., 30 °C (Luck et al., 1993), but a better method is to feed infected females with antibiotics such as tetracycline or rifampicin, and then examine whether the egg hatch rate and the proportion of male progeny increases (Stouthamer et al., 1990, 2002; Hurst et al., 1992). Alternatively, one can stain eggs or haemolymph cells with the DNA stains DAPI or H33258 and examine them under the microscope for the presence of bacteria using cured strains as controls. Typically, hundreds of bacteria occur in a single egg (Breeuwer & Werren, 1990; Stouthamer & Werren, 1993; Hurst et al., 1996). Majerus (2001) and Tinsley and Majerus (2006) demonstrated that male-killing can be elicited by artificial horizontal transfer via microinjection, into uninfected hosts, which then exhibit male-killing.

Cytoplasmic Incompatibility

Another cause of sex ratio distortion is cytoplasmic incompatibility caused by *Wolbachia* bacteria. In several parasitoids (Luck et al., 1993; Werren & O'Neill, 1997; Stouthamer et al., 2002), strains are found that harbour *Wolbachia* (Sect. 3.3.3). These bacteria are present in the egg cytoplasm. Successful fertilisation of eggs free of the bacterium can only be achieved by sperm from uninfected males, whereas eggs containing the bacterium can be fertilised by sperm from either infected or uninfected males (Breeuwer & Werren, 1990). In addition to this unidirectional cytoplasmic incompatibility, bi-

directional incompatibility has been observed. This refers to the situation where two strains harbour different types of *Wolbachia* and both reciprocal crosses between strains are incompatible. The sperm chromosomes are destroyed in the fertilised egg in incompatible crosses (Breeuwer & Werren, 1990). Whereas this leads to no progeny at all in diploid organisms (because haploid eggs are inviable), it results in all-male broods in haplodiploids.

Wolbachia-Mediated Thelytoky

Thelytoky in some parasitoid wasps is caused by *Wolbachia*. Stouthamer et al. (1990) demonstrated that thelytokous *Trichogramma* females began to produce sons after feeding on antibiotics. Similar reversion of thelytoky to arrhenotoky has been reported for a number of other species (reviewed in Stouthamer, 1997).

Other cases of thelytoky in parasitoid wasps may also be induced by micro-organisms. Several studies have found an increase in male production of thelytokous strains after exposing them to high temperatures (e.g., Wilson & Woolcock, 1960; Legner, 1985). They may revert to thelytoky when reared at lower temperatures. This is consistent with the hypothesis that the induction of thelytoky is dosage dependent, i.e., high temperatures kill the bacteria and reduce their density to below a critical value, but their numbers may recover (Breeuwer & Werren, 1993; Hurst, 1993).

Feminisation

Wolbachia have also been reported to cause feminisation, i.e., change genotypic males into females. The best-studied case is that of the isopod *Armadillidium vulgare* (Rigaud, 1997; Cook, 2002), but it has also been reported from two insects, the Asian corn borer, *Ostrinia furnacalis* (Kageyama et al., 1998) and the leafhopper *Zyginidia pullulan* (Negri et al., 2006). There are limited reports from insect natural enemies, however, Ma et al. (2015) show that thelytoky in the parasitoid wasp *Asobara japonica* is a two-step process of diploidisation and feminisation of host eggs. Hence, researchers working with such insects should be aware of the possibility.

3.5 Quantitative Genetics

3.5.1 Introduction to Quantitative Genetics

Quantitative genetics studies the inheritance of traits that are of degree rather than of kind (Falconer & Mackay, 1996). Quantitative traits (QTs), e.g., body-size, usually show a continuous distribution of values that is approximately Gaussian. Understanding the genetics of QTs is essential for the study of any biological system because the majority of morphological, physiological, behavioural and life-history traits have quantitative characteristics.

The genetics underlying quantitative traits is often assumed to be polygenic: differences at many loci, each with a small effect, contribute to the genetic variation for the trait in the population under study. It is important to realise, however, that as few as three loci can produce normal distributions very much like the distributions shown by QTs (Thoday & Thompson, 1976). The implication of the underlying genetics, whether oligogenic (a few genes) or polygenic (many genes), and the continuous distribution of QTs, is that the individual genes cannot be identified by their phenotypic segregation, hence Mendelian analysis does not apply. Moreover, QTs usually depend strongly on the environment in which the trait is measured or in which the organism has developed, or both. Below, how the genetic analysis of QTs has taken these aspects into account by using variance analysis and parent–offspring resemblance is discussed. Apart from classical quantitative genetics, the possibility of using linkage maps to locate the actual genes underlying QTs, quantitative trait loci (QTL) mapping, is described.

3.5.2 Classical Quantitative Genetics

This section provides a brief outline of the principles of quantitative genetics, but for a more thorough introduction the reader is referred to a variety of textbooks such as those by Falconer

and Mackay (1996), Kearsey and Pooni (1996), Lynch and Walsh (1998) and Roff (1997). Quantitative traits are expressed in values. The phenotypic value (P) is the trait value measured for an individual. It is the sum of the genotypic value (G) and the environmental effect (E) (Falconer & Mackay, 1996):

$$P = G + E \tag{3.5}$$

The phenotypic value of an individual can be measured relative to the population mean. The population mean itself is expressed in terms of allele frequencies, genotypic values and the degree of dominance (population mean equals $a(p-q) + 2dpq$). Consider a locus with two alleles, A_1 with frequency p and A_2 with frequency q. We can assign values to the three possible genotypes A_1A_1, A_1A_2 and A_2A_2. Let this genotypic value for A_1A_1 be a and for A_2A_2 be $-a$. In the case that the alleles act additively, the genotypic value of A_1A_2 would be $((a + (-a))/2)$ equals zero. When there is dominance, the genotypic value would be different from zero. The genotypic value of the heterozygote A_1A_2 is usually called d (Fig. 3.18). Therefore, d depends on the level of dominance and this is often expressed as the ratio d/a (Falconer & Mackay, 1996). Note that the genotypic value can be measured, but this is only practically possible when dealing with a single-locus situation in which all genotypes can be phenotypically distinguished.

An important concept in quantitative genetics is the breeding value of an individual, which can be measured by the mean phenotypic value of its progeny (Falconer & Mackay, 1996). Therefore, the breeding value is the sum of the average effects of the parents' genes on their progeny and this is what most researchers are interested in. Breeding values are a property of the gene and the population and depend on the degree of dominance and the allele frequencies. Only in the absence of dominance are breeding values and genotypic values the same.

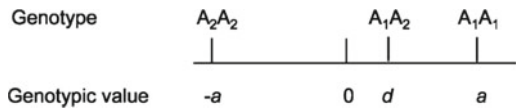


Fig. 3.18 Schematic depiction of genotypic values for a one-locus-two-allele situation. a measures the additive effect and d is a measure of the dominance effect. When $d = 0$ the alleles are completely additive, when $d = a$, A_1 is completely dominant over A_2 and when $d = -a$, A_2 is completely dominant over A_1

Variances

As mentioned above, QTs show a continuous distribution of phenotypic values with a more or less normal distribution. In principle, the amount of variation around the population mean reflects both the effects of genes for the trait as well as the effect of the environment on the trait. Partitioning of variance components will show the relative importance of genetics and hence the potential for (natural) selection and/or evolution of the trait in that particular population. The total phenotypic variance (V_P) is:

$$V_P = V_G + V_E \tag{3.6}$$

where V_G and V_E are the genetic variance and the environmental variance, respectively. The genetic variance is partitioned into the additive (V_A) and interaction components (within gene: the dominance component V_D; between genes: the epistatic component, V_I, that measures the variance due to interaction between genes). Hence:

$$V_P = V_A + V_D + V_I + V_E \tag{3.7}$$

By definition, additive effects are independent of the genetic background in which the alleles in question are placed. In contrast, the effect of alleles that show within and/or between gene interaction depends on the genotype in which they occur. Since parents pass on their genes and not their genotypes, the additive genetic component is the most significant if one wants to predict the outcome of short-term selection on the trait and/or evolution of the trait. That is not to say the other components should be ignored. Dominance and epistatic variation can be converted into additive genetic variance due to bottlenecks or drift (Cheverud & Routman, 1996; Roff, 1997). In addition, studying the ratio between V_D and V_A is important, for example, for

the study of the mechanisms behind the maintenance of genetic variation (Charlesworth, 1987).

Heritability

The heritability (h^2) is the ratio between the genetic variance and the total, phenotypic, variance. Hence it measures the degree to which a QT is genetically determined in a population. Two h^2 measures are distinguished, the broad-sense h^2:

$$h^2 = V_G/V_P = (V_A + V_D + V_I)/V_P \qquad (3.8)$$

and the narrow-sense h^2,

$$h^2 = V_A/V_P \qquad (3.9)$$

Following the reasoning above, the QT's narrow-sense h^2 is the most informative about the potential for selective responses in the QT.

The relationship between more than one QT is measured as the correlation between two traits. The genetic correlation uses the covariance between two traits and can be established using several experimental designs (see below and Roff, 1997). Genetic correlations are an important issue in evolution in general and for the evolution of life histories in particular. Negative genetic correlations between traits, or so-called trade-offs, are especially important because they can impose (short-term) constraints on adaptive evolution in populations: a trait value cannot increase or decrease without a concomitant decrease or increase in the other, negatively correlated, trait.

The above shows that the genetics of QTs can be satisfactorily described in terms of statistical variances. However, a major caveat associated with QTs is their strong environmental subjectivity. This extends to both the general effect of the environment on the individual's phenotypic trait value, as well as the differential response of genotypes to environmental change, a phenomenon known as gene–environment interaction. The careful reader will have noticed that the $G \times E$ interaction should be added to the variance components. However, usually, this component can be ignored (Falconer & Mackay, 1996; Lynch & Walsh, 1998). The general effect of the environment will seriously bias estimates of genetic variances and heritabilities. When, for instance, the rearing conditions are poor, it is likely that several morphological, physiological and life-history traits will be negatively affected. The phenotypic variance could then be increased, hence h^2 estimates would be deflated and any genetic component to V_P would be underestimated. Therefore, estimates of variance components and h^2 apply only to the environment in which they are measured and any extrapolation outside this environment should be carried out with caution. In evolutionary ecology, heritability estimates should ideally be obtained in the field. Such estimates have been reported to be lower in the field as compared to controlled laboratory conditions as a result of e.g., increased V_P (Hoffmann & Schiffer, 1998) or a decrease in V_A and increase in V_P (Simons & Roff, 1994). Surprisingly though, an extensive literature review showed that the h^2 estimates of morphological and life-history traits were higher in the field than in the laboratory, but that the reverse is true for behavioural traits (Weigenberg & Roff, 1996; Roff, 1997). Moreover, in these studies, a strong correlation existed between field and laboratory estimates of h^2. This general trend implies that the combination of effects on V_A and V_P should be measured. It also suggests that gene–environment interactions may affect V_G and V_A specifically. Note that V_G does not measure which gene is contributing to the genetic variance. Consequently, although genetic variance estimates can be similar, very different genes may contribute to this variance. Studying $G \times E$ interaction is an interesting field in itself and has many implications for theories of evolution, such as the maintenance of genetic variation (Gillespie & Turelli, 1989).

Experimental Designs

Several experimental designs can be used to estimate the quantitative genetic parameters mentioned above. The concepts above and below

are all based on the genetic relationship between relatives, or the likelihood that they share the same alleles. The degree to which a trait is associated between two individuals (the covariance) is generally given (ignoring epistatic interactions) by:

$$\text{cov} = rV_A + uV_D \tag{3.10}$$

with r the probability of sharing the same allele, and u the probability of having the same genotype for a locus (Margolies & Cox, 1993; Falconer & Mackay, 1996). Obviously, the covariance depends on the genetic system that determines the relatedness between individuals and is therefore very different for diploid and haplodiploid species (Margolies & Cox, 1993). Many insect natural enemies are parasitoid wasps, which are haplodiploid, hence the experimental designs will be discussed for both genetic systems.

Full-Sib Design

This experimental design is very often used because it can be implemented in all biological systems. Single crosses are set up between a female and a male and the resulting families are raised separately. For the diploid situation, the covariance will be:

$$\text{cov}_{\text{FSD}} = \frac{1}{2}V_A + \frac{1}{4}V_D \tag{3.11}$$

where FSD means full-sib diploid. This can be reasoned as follows: for a cross between A_1A_2 and A_3A_4, the four resulting progeny genotypes are A_1A_3, A_1A_4, A_2A_3 and A_2A_4. Comparing any one genotype with all four possible genotypes (full-sib comparisons) shows that on average in half of the cases an allele is shared and in one quarter of the cases the genotype is shared.

For the haplodiploid situation, males and females must be discriminated. Males are haploid, therefore by definition the within-gene interaction (V_D) is absent. The males will, on average, share half their alleles with each other, r = 0.5, hence (using Eq. 3.10),

$$\text{cov}_{\text{FSHM}} = \frac{1}{2}V_A \tag{3.12}$$

where FSHM means full-sib haploid male and FSHF (below) means full-sib haploid female. For females, the cross can be given as between A_1A_2 and A_3. The females in the progeny will be either A_1A_3 or A_2A_3. Therefore, following the reasoning above, r = 3/4 and u = 1/2, hence (using Eq. 3.10),

$$\text{cov}_{\text{FSHM}} = \frac{3}{4}V_A + \frac{1}{2}V_D \tag{3.13}$$

An important point to notice in the full-sib design is that only for the haplodiploid males can the additive genetic variance be estimated. For all the other cases, estimates of genetic variance include the dominance deviation, hence heritability estimates will all be broad sense. A full-sib design has successfully indicated genetic variation for oviposition behaviour in *Trichogramma maidis* (Chassain & Boulétreau, 1987), but the full significance of the variation for selective changes in nature remains obscure without knowing the individual components of this variation. See Wajnberg (2004) for an exhaustive review of studies carried out to quantify genetic variation in parasitoid wasps.

A technique that is often applied in parasitoid wasps is the use of iso-female lines (Parker & Orzack, 1985; Wajnberg et al., 1985, 1989, 1999, 2004, 2012; Boulétreau & Wajnberg, 1986; Antolin, 1989; Prévost & Lewis, 1990; Bruins et al., 1994; Cronin & Strong, 1996; Kraaijeveld et al., 1998; Perez-Maluf et al., 1998; Wajnberg & Colazza, 1998; Sevarika et al., 2021; Sect. 3.2.3). The main reason for this is, perhaps, that using the asexual production of males and then back-crossing it to the mother (Kraaijeveld et al., 1998) is an easy way of obtaining near-isogenic lines. The genetic variance is then estimated from the between-lines mean squares in an analysis of variance. Again, this procedure estimates the V_G and broad-sense heritabilities. Cronin and Strong (1996) discuss the

possibilities of natural and artificial selection using data from isogenic lines. However, their conclusions may be confounded by the lack of knowledge of the individual genetic variance components. Another potential problem with using iso-female lines is the interpretation of correlations between traits. Using iso-female lines will bias towards positive genetic correlations, because the best line (genotype) is best in everything and linkage between genes is mistaken for pleiotropic action of single genes.

A full-sib design has also been used to study phenotypic plasticity in adult abdominal colour pattern in the hover-fly *Eristalis arbustorum* (Ottenheim et al., 1996). Offspring of single females were reared at six different temperatures, ranging from 8 to 26 °C, and significant variation between females was found for the slope of colour pattern on temperature. This indicated genetic variation for plasticity.

Half-Sib Design

The problem of the broad-sense genetic variance component can be circumvented using a half-sib design. For biological reasons, usually a male (sire) is mated to several females (dams) and this is done for a large number of males. Half-sib designs are more difficult to perform because they require a biological system where multiply-mating males are easily obtainable.

For the diploid situation, consider a male, A_1A_2, that has been mated with two females, A_3A_4 and A_5A_6. The resulting progeny of these families are A_1A_3, A_1A_4, A_2A_3 or A_2A_4, and A_1A_5, A_1A_6, A_2A_5 or A_2A_6, respectively. Comparing any genotype from one family with all the genotypes from the other family (half-sib comparison), shows that on average in one-quarter of the comparisons an allele is shared and in no case is a genotype shared ($r = 1/4$, $u = 0$). Hence (using Eq. 3.10):

$$\mathrm{cov}_{\mathrm{HSD}} = \frac{3}{4} V_A \tag{3.14}$$

The haplodiploid cross can be exemplified as, A_1 (male) crossed with A_2A_3 and A_4A_5 (females). The resulting genotypes of the progeny are A_2 or A_3 (males) and A_1A_2 or A_1A_3 (females), and A_4 or A_5 (males) and A_1A_4 and A_1A_5 (females), respectively.

Clearly, half-sib males do not share any allele and can therefore not be used to estimate genetic variance components. For females, half-sibs share half of their alleles, but never the genotype ($r = 1/2$, $u = 0$). Hence (using Eq. 3.10):

$$\mathrm{cov}_{\mathrm{HSHF}} = \frac{1}{2} V_A \tag{3.15}$$

Note that in all cases the additive genetic variance component can be directly estimated. Table 3.2 summarises the results and gives a practical guide to the interpretation of the nested analysis of variance (as performed by most statistical computer packages) and to the calculations of the variance components. It is clear that in a half-sib design V_D can also be estimated. However, without replication, there is no direct test of its significance (Kearsey & Pooni, 1996).

A half-sib design was conducted to study the cost of chemical defence in the two-spot ladybird, *Adalia bipunctata* (Holloway et al., 1993). All characters measured showed high levels of additive genetic variances. No clear negative covariances were found between the life history characters body-weight and growth-rate. This was thought to be due to sex-dependent gene expression.

Another point of interest to raise is the environmental variance. This variance can be partitioned into a general environmental effect (V_{Ew}) and an environmental effect arising from siblings being raised in a common environment (V_{Ec}) (Falconer & Mackay, 1996). For the analysis of variance (the resemblance between relatives), only the latter component is important because it can cause similarities between unrelated groups that are not the result of genetic differences between the groups but are due to the common environment that individuals within each group share. Careful experimental design can take care of this, by rearing members from each family in more than one group and/or randomising members over the rearing environment (Falconer & Mackay, 1996).

Table 3.2 Nested analysis of variance for a half-sib design with n males mated with m females and each family containing r progeny (where n, m, and r, represent the number of males, females and progeny, respectively). (**A**) ANOVA table with source of variation, degrees of freedom (df), mean squares (MS) and expected mean-squares components (after Kearsey & Pooni, 1996). (**B**) The relationship between causal and mean-squares components (after Margolies & Cox, 1993)

A

Source	df	MS	Components
Between male half-sib family groups	$n - 1$	MS_M	$\sigma^2_W + r\sigma^2_{F(M)} + rm\sigma^2_M$
Between full-sib families within male half-sib family group	$n(m - 1)$	$MS_{F(M)}$	$\sigma^2_W + r\sigma^2_{F(M)}$
Within full-sib families	$nm(r - 1)$	MS_W	σ^2_W
Total	$nmr - 1$		

B

Components	Covariance estimate	Causal components	
		Diploid	Haplodiploid
σ^2_M	cov_{HS}	$^1/_4V_A$	$^1/_2V_A$
$\sigma^2_{F(M)}$	$cov_{FS} - cov_{HS}$	$^1/_4V_A + {}^1/_4V_D$	$^1/_4V_A + {}^1/_2V_D$
σ^2_W	$V_P - cov_{FS}$	$^1/_2V_A + {}^3/_4V_D$	$^1/_4V_A + {}^1/_2V_D$
$\sigma^2_W + \sigma^2_{F(M)}$	cov_{FS}	$^1/_2V_A + {}^1/_4V_D$	$^3/_4V_A + {}^1/_2V_D$
$\sigma^2_W + \sigma^2_{F(M)} + \sigma^2_M$	V_P	$V_A + V_D$	$V_A + V_D$

Test Crosses

The above-described half-sib design is known as the North Carolina Experiment I (NCI) (Kearsey & Pooni, 1996). Several varieties exist, such as the NCII, in which each male is crossed to each female. Naturally, this can only be done if the lines used in the crosses are (near)-isogenic. In this design the interaction variance between female and male half-sib families can be used to directly test for the significance of V_D (Kearsey & Pooni, 1996). Using triple test crosses (TTCs) the between-gene epistatic interaction component (and in turn its components, like dominance-by-dominance interaction) can also be estimated. It can be used if one has highly inbred or isogenic lines and involves back-crossing of F_1 and F_2 progeny from two parental strains, to both the parental strains and their F_1 (for F_2 progeny). Discussing these complex designs (note for instance that four possible F_2 progeny can be made) in full is beyond the scope of this chapter: Kearsey and Pooni (1996) provide an excellent introduction to this subject.

Parent–Offspring Regression and Realised Heritability

Heritabilities can be calculated whenever broad or narrow-sense genetic variation estimates are available (Eqs. 3.8 and 3.9). There are two additional ways to estimate h^2, using parent–offspring regression and realised heritabilities.

Parent–offspring regression is relatively straightforward. The phenotypic value is measured, preferably, for both parents and their offspring. The values of the progeny are subsequently regressed on the values of the parents (using many families as individual data points). The slope (b) of the regression line is an estimate for h^2 (Falconer & Mackay, 1996).

The regression can be done using the mid-parent values and the average of the progeny. In that case, for diploids:

$$h^2 = b \tag{3.16}$$

Alternatively, values of the sons can be regressed on the fathers, and values of the daughters on the

mothers. Since, in the diploid case, fathers and sons, and mother and daughters on average only share half their genes:

$$h^2 = 2b \tag{3.17}$$

If differences between son-father and daughter-mother regressions are apparent, it is an indication of maternal effects or genomic imprinting influencing the trait under consideration. For haplodiploid species, "son" and "father" regression is not possible because they do not share any genes (see above), whereas the daughter-mother regression is identical to that for diploids. Parent–offspring regression for sex ratio traits in *Nasonia vitripennis* showed a heritability of around 0.10 (Orzack & Gladstone, 1994).

The selection response (R) in an experiment depends on the selection differential (S) between the mean value of the population and the mean value of the selected group, and the heritability of the trait:

$$R = h^2 S \tag{3.18}$$

Hence, knowing the selection response and the selection differential, the heritability can be estimated. This can be done in a one-generation experiment, but the so-called "realised heritability", is usually calculated over several generations. The selection response is regressed on the cumulative selection differential over the generations, thus giving the slope of the regression line as a direct estimate of h^2. The response should be measured against a certain control line, to account for the between-generation general environmental effects. This estimation procedure assumes that the additive genetic variance remains unchanged over the experiment. When selection is successful this is obviously not the case. Therefore, realised heritabilities should be estimated over the first part of the experiment where this assumption is less likely to be violated. In haplodiploid species this h^2 estimate should be multiplied by a factor 3/2, because fewer genomes contribute to the selection response (Bulmer & Bull, 1982). The adjusted realised heritability for sex ratio in *Nasonia vitripennis* was on average 0.12 (Parker & Orzack, 1985). No selection response was found, however, in a similar experiment for sex ratio in *Trichogramma fasciatum* (Ram & Sharma, 1977).

General Considerations

Estimation of genetic variance components and heritabilities is notoriously difficult. In general, large sample sizes are needed, and, depending on the questions one wants to answer (Houle, 1992), the number of sires, dams, or progeny per dam should be optimised (Roff, 1996, 1997). The haplodiploid system requires fewer individuals to be measured than the diploid system to achieve the same level of efficiency, because r (the probability that individuals share the same allele) is greater in the former (see above). For the half-sib design, using a haplodiploid species requires half as many females per male, as compared to using a diploid species, because $r = 1/2$ (Eq. 3.15) and $r = 1/4$ (Eq. 3.14), respectively (Margolies & Cox, 1993).

According to population genetic theory, little additive genetic variation for total fitness exists in populations, above the amount generated by mutation. This is generally corroborated by the observation that life-history traits have lower heritabilities than morphological traits, with heritabilities of behavioural and physiological traits somewhere in between (Mousseau & Roff, 1987; Roff, 1997). Trade-offs between traits and $G \times E$ interactions for traits can, among other factors, significantly promote the maintenance of genetic variation (Roff, 1992; Stearns, 1992; Lynch & Walsh, 1998). As reviewed by Kraaijeveld et al. (1998) and discussed below, trade-offs in host–parasite systems play an important role in balancing the costs and benefits of resistance to the parasitoid, thus maintaining the genetic variation in resistance and virulence as observed between (geographical) distinct populations and in selection experiments. The potential genetic complexity of QTs closely related to fitness and the genetic correlations between the

QTs are important aspects to study in order to further our understanding of host–parasite systems and are essential for implementing successful biocontrol programmes (see also Antolin, 1989; Wajnberg, 2004).

3.5.3 Genetics of Host–Parasite Interactions

The potential for co-evolution is particularly high in host–parasitoid systems. Parasitism directly affects the survival of the host's offspring (eggs, larvae or pupae) which is greatly reduced, often to zero. As a consequence, the reproductive success of the host is greatly reduced. Therefore, selection is strong for genetic variants that increase the survival of offspring either through avoidance or through resistance against the parasitoid. In turn, the reproductive success of the parasitoid directly depends on the survival of its eggs in the host. Hence, mutants with offspring that (more) efficiently circumvent the host's defence systems, i.e., are more virulent, will have a high(er) reproductive success. In summary, the reciprocal selection pressures on the parasitoid and the host are high and may result in a 'tit for tat' accumulation of adaptations and counter-adaptations.

A prerequisite for natural selection and co-evolution is the existence of genetic variation for both virulence and resistance. A first indication of the presence of such genetic variation comes from virulence and resistance differences between strains from different geographical regions. Especially if such variation shows longitudinal or latitudinal clinal patterns, it can be taken as evidence that different selection pressures are operating in the different populations. Moreover, reciprocal (genetic) interactions between hosts from one region and parasites from the other will indicate co-evolutionary selection and local adaptation. Geographical variation has been found for the virulence of the parasitoid *Asobara tabida* (Fig. 3.19) and the resistance of its host *Drosophila melanogaster* (Kraaijeveld & van Alphen, 1995). In this system

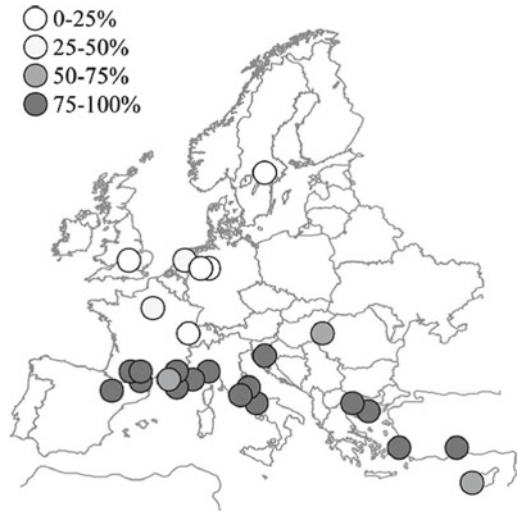


Fig. 3.19 Geographical variation within western Europe in avoidance of encapsulation of the parasitoid *Asobara tabida* by a reference strain of its host *Drosophila melanogaster*. *Source* Kraaijeveld and van Alphen (1994). Reproduced by permission of Cambridge University Press

the most important factors determining this variation were considered to be the presence or absence of alternative hosts, and competition by other parasitoid species. Furthermore, only one reference strain of *D. melanogaster* was used, which prevents any definite conclusions about local adaptation and co-evolution (Kraaijeveld et al., 1998). As shown below, local adaptation has been found in the *Leptopilina boulardi–Drosophila melanogaster* system (Carton & Nappi, 1991).

The Traits

Host: Avoidance and Resistance

Hosts can reduce the cost of parasitism both through avoidance and resistance. Avoidance can be achieved by changes in the host's behaviour. For instance, larval activity could be reduced to avoid parasitoids that rely on vibrotaxis, or larvae can feed deeper into the substrate to avoid the parasitoid's ovipositor.

Resistance can involve both humoral and cellular responses. Some instances of humoral

responses have been studied (see references in Kraaijeveld et al., 1998) but the cellular response is much better investigated both at the physiological and molecular level. Immunology underlies the cellular response, such that certain cells (haemocytes) in the host's haemocoel attach to the foreign parasitoid egg and the continuing aggregation of such cells to the egg results ultimately in the death of the egg. This mechanism of encapsulation is a general defence mechanism seen in all arthropods (Sect. 2.10.2).

Parasite: Avoidance and Resistance

The strategy of the parasitoid for counteracting the host's defence mechanisms has also been divided into avoidance and resistance traits, although in some cases this distinction is not very transparent. Avoidance can be considered a passive strategy and refers to eggs that are not in contact with the haemolymph, such as eggs laid in tissue that is devoid of haemocytes or that cannot be reached by haemocytes. Ectoparasitoids also practice avoidance (Askew, 1971).

Resistance is considered an active strategy for eluding the host's defences. Eggs are in contact with the haemolymph. At oviposition, the parasitoid female can inject substances that paralyse the immune responses. For example, injection of teratocytes by the female serves to deplete haemocytes by deflecting encapsulation from the egg to the teratocytes. Another resistance strategy is to lay more than one egg per host to quickly deplete the number of haemocytes. It has been shown for *Asobara tabida* that an egg laid in a *Drosophila* larva parasitised by *Leptopilina boulardi* has a higher chance of survival than one laid in an unparasitised host (Kraaijeveld, 1999). Other examples of resistance in the braconid *Asobara tabida*, are fast-growing eggs that outcompete haemocytes that are not present in sufficient numbers to encapsulate the growing egg/embryo (Mollema, 1988) and sticky eggs that attach to host tissue which protects them from complete coverage by haemocytes (Kraaijeveld & van Alphen, 1994; Monconduit & Prévost, 1994). Finally, molecular mimicry has also been described, in which the parasitoid egg is recognised as "self" instead of "foreign" (Feddersen et al., 1986).

Intriguingly, other parasitoids use symbiotic association with viruses to attack the immune system of the host. The viruses are either securely integrated in the parasite's genome or end up in the parasite because of feeding in the host. The integrated viruses only replicate in the female parasitoid prior to oviposition. Other parasitoids use virus-like particles (VLPs, e.g., *Leptopilina boulardi*) to counteract the host immune reaction. VLPs have structures that superficially resemble viruses but that lack nucleic acids (Quicke, 1997). VLPs are used in several ways by the parasitoid. They can form a shield around the parasitoid egg in order to make it invisible to the host immune system (e.g., *Venturia canescens*). This is a form of molecular mimicry. The VLPs are coded for by the parasitoid genome and, for *Venturia*, the gene has been cloned (Theopold et al., 1994). VLPs can also be used to directly attack the haemocytes. For instance, in *Leptopilina heterotoma*, VLPs enter the lamellocytes (haemocytes found only in larvae, that function to encapsulate parasites and pathogens) and change the morphology and surface characteristics of these cells such that encapsulation is blocked (Rizki & Rizki, 1990). Schlenke et al. (2007) reviewed progress and investigated the ability of *L. heterotoma* and *L. boulardi* strains to evade the *Drosophila* host immune response. Lee et al. (2009) used analysis of changes in gene expression to identify immune and physiological responses between *Drosophila* and *Leptopilina* species. Heavner et al. (2017) investigated VLPs in *L . heterotoma* and identified that they contain a mix of prokaryotic and eukaryotic properties. The authors proposed the term mixed-strategy extracellular vesicle (MSEV) to replace VLP.

Practical Aspects

Measuring Avoidance and Resistance

The traits involved in host and parasitoid avoidance and resistance are most accurately measured under controlled conditions. This

usually entails rearing both the host and the parasitoid in laboratory situations. Most studies that measure the abilities of the host and the parasitoid use a single reference strain (Kraaijeveld et al., 1998). For instance, if one wishes to assess the ability of parasitoids from different populations to successfully find the host and/or circumvent a host's defences, the parasitoids are supplied with hosts from a single strain that has been reared under controlled conditions (e.g., Kraaijeveld & van Alphen, 1994). Such reference strains are usually genetically (near-)isogenic to avoid any possible variation in parasitoid performance caused by genetic variation in the host.

However, there is a serious problem with using a single, genetically uniform, reference strain: gene–environment interactions. This problem arises if the individuals that are being compared are reared in an environment to which they are not equally suited (Stearns, 1992). In this case, any differences found between the parasitoids in the above example may well be spurious. This is particularly a problem when one intends to measure negative genetic correlations, i.e., trade-offs (Partridge, 1989). Moreover, (near-) isogenic, hence inbred, lines may have reduced performance with respect to the trait(s) of interest. Although this may not affect the relative ability scores of the parasitoids, it will certainly affect the absolute score values. As mentioned above, experimental conditions should be controlled. This does not mean that no varying conditions should be applied, but those that are applied should be applied in a controlled manner. Hence, to avoid the problems associated with the use of a single reference strain, more host strains should be used in order to investigate host source and parasitoid source interactions. This could be combined with research on local adaptation. For instance, Carton and Nappi (1991) reported that a central African population of *Leptopilina boulardi* was not successful in evading the host encapsulation defence. The parasitoid tested against the sympatric host population showed that 42% of the eggs were encapsulated. However, when tested against four allopatric host populations, encapsulation percentages ranged from 49 to 78. This is a clear example of local adaptation of the parasitoid to its host. This would have been missed had a single host strain been used.

As with so many aspects of host–parasite/parasitoid interactions, molecular biology is increasingly being used to investigate the processes. Wertheim et al. (2011) assessed changes in gene expression, in a *Drosophila melanogaster* strain artificially selected for resistance against *Asobara tabida*, to investigate whether responses were due to evolutionary adaptation or phenotypic plasticity. Wan et al. (2021) identified changes in several cell types involved in processes such as encapsulation and melanisation of parasitoid eggs and other networks and components of the host immune system. Such findings and the techniques used will, without doubt, lead to greater understanding of these interactions in many systems.

Genetic Aspects

In principle, all standard genetic tools can be employed to study the genetics of host and parasitoid traits. To date, most investigations have used analysis of iso-female lines, selection experiments and line crosses, and segregation analysis to estimate genetic variation. In addition, Henter (1995) used a half-sib design to further partition the genetic variances for the virulence of *Aphidius ervi*. It was shown that 26% of the phenotypic variation was due to additive genetic factors. In general, the estimates of genetic variance and heritability (Sect. 3.5.2) were moderately high (0.2–0.7; Kraaijeveld et al., 1998). This shows that there is scope for selection on both the parasite and the host traits.

The genetic basis of virulence and resistance traits was relatively unexplored until molecular genetic and next-generation sequencing technologies became more widely available and affordable. As mentioned, quantitative genetic tools have been employed because traits such as encapsulation responses can be considered to be quantitative. However, a quantitative trait does not necessarily imply that it is polygenically

determined with many loci with small effect. In fact, the ability to encapsulate might well be determined by a single gene or a small number of genes.

Most early studies tended to conclude that encapsulation has a polygenic basis with the evidence resulting from line crosses (reviewed by Kraaijeveld et al., 1998). Analysis of line crosses is subject to substantial error, e.g., when sample sizes are low (Lynch & Walsh, 1998; Roff, 1997). To analyse fully these instances, line crosses should be used in combination with gene mapping (Sect. 3.5.4). In contrast evidence is mounting that the traits involved in several host–parasitoid systems are determined by major genetic factors.

One striking example of a single gene is the Sitter/Rover polymorphism that affects the moving behaviour of the host *Drosophila melanogaster* (Sokolowski, 1980). Rover larvae actively move through the medium, while the Sitter larvae are more sessile. The Rover allele is dominant over the Sitter allele. This genetic polymorphism has major consequences for the likelihood of being parasitised, e.g., by *Asobara tabida* (Kraaijeveld et al., 1998). More recent studies by Anreiter et al. (2017) show that epigenetic modification of gene expression underlies these differences in behaviour.

In the *Leptopilina boulardi-Drosophila melanogaster* system, heritabilities were measured using the iso-female line procedure (Carton & Nappi, 1991). The heritability for encapsulation was estimated at 0.43, while the heritability for evading the encapsulation was estimated between 0.3 and 0.5. On the basis of these data, one would be inclined to conclude that both traits have a polygenic basis. However, analysis of crosses between susceptible and resistant isogenic *Drosophila* lines (two parental line crosses, two reciprocal F_1 hybrids, eight reciprocal backcrosses and four reciprocal F_2 hybrids) strongly indicated that encapsulation is a monogenic trait with resistant being dominant over susceptible (Carton & Nappi, 1991). Subsequently, this was confirmed in a detailed genetic study (Poirié et al., 1999). The resistance *Leptopilina boulardi* gene was named *Rlb* and work moved to cloning the gene. To this end, detailed mapping experiments (Sect. 3.5.4) were performed using *Drosophila melanogaster* deletion strains (Lindsley & Zimm, 1992) and the avirulent strain of *Leptopilina boulardi* described above (Carton & Nappi, 1991). A detailed physical map was obtained in the region 55C;55F3 (especially 55E2-E6;F3; Carton & Nappi, 1997; Poirié et al., 1999). Interestingly, in the encapsulation response of *Drosophila melanogaster* against *Asobara tabida* parasitism appeared also to be determined by a major gene (*Rat*, resistant to *A. t abida*, resistant allele is dominant; Poirié et al., 1999, 2000). *Rat* was located 35 cM away from *Rlb*, but also on the second chromosome. It would be useful to study the polymorphism on *Rat* in relation to the clinal variation found in the encapsulation response of *Drosophila melanogaster* discussed above (Kraaijeveld & van Alphen, 1995). The apparent monogenic nature of the *Drosophila melanogaster* encapsulation response fits well with the interpretation of selection experiments for increased resistance in this species (Kraaijeveld et al., 1998).

The genetics of resistance in *Leptopilina boulardi* has also been studied in more detail. Analyses of crosses between the African avirulent strain and a virulent strain indicated that the immune suppressive ability had a monogenic basis with incomplete dominance of the resistant allele (Dupas et al., 1998). There was also evidence for the presence of minor genes involved in the resistant phenotype. These minor genes may well underpin the local adaptation of the African *Leptopilina* population to its sympatric host (see above). Further studies on the genetic interactions between *L. boulardi* and *Drosophila yakuba* identified that unlike the "gene-for-gene" type of interaction between *D. melanogaster* and *L. boulardi*, where parasitism succeeds unless the parasite has no virulence alleles and the host has at least one resistance Rlb^+ allele, in *D. yakuba* the parasitoid egg is encapsulated, unless the parasite is homozygous for the virulence alleles and the host homozygous for the susceptible alleles (Dubuffet et al., 2007). Subsequently, extensive molecular genetic investigations have demonstrated that there are complex genotype-

by-genotype interactions between the *Drosophila* host and its parasitoid (Leitão et al., 2019). The publication of the *L. boulardi* genome (Khan et al., 2020), will provide further valuable insight into these and many other questions around the genetic and epigenetic control of the relationship between this parasitoid and its hosts.

One further system offers potential for harnessing the complex interactions of host, symbiont and parasitoid, to increase the efficacy of biological control. Many aphids are infected with the endosymbiont *Hamiltonella defensa*, which enhances the aphid resistance to parasitoids and hence reduces the effectiveness of the parasitoids in controlling the aphids. Rossbacher and Vorburger (2020) demonstrated that experimental evolution, via pre-exposure to aphids infected with *H. defensa,* allowed the parasitoids to more effectively control the aphid populations. It is suggested that the mechanism involves a toxin-encoding bacteriophage called *Acyrthosiphon pisum* secondary endosymbiont (APSE) in *H. defensa'*s genome (Oliver et al., 2009; Brandt et al., 2017).

As a final note, there may be a causal link between the genetic methods used and the genetic basis found in some of the experiments discussed above. Single iso-female or isogenic lines as used by Carton and Nappi (1991) are biased towards single gene effects (monogenic); they do not reflect the actual amount of genetic variation in the source population. Kraaijeveld and Godfray (1997) used (family) selection as a method to uncover the genetic basis of resistance. Their approach is more likely to pick up major and minor genes (polygenic) because it samples from the total amount of genetic variation available in the population. Moreover, the resistance and virulence phenotypes are a combination of several traits, which all have different genetic bases.

Maintenance of Genetic Variation: Costs and Trade-Offs

The quantitative genetic parameters, such as genetic variances and heritabilities, for resistance and avoidance traits in hosts and parasitoids, indicate high levels of genetic variation. Independent of whether the genetic basis is monogenic or polygenic, this implies that in nature polymorphisms exist for the traits. This suggests that balancing factors are at play. Resistance to either parasitoid attacks or host defences is under strong selection because of the direct fitness benefits. The counteracting force could be the cost of resistance and/or avoidance. The cost and benefit trade-off and the balance between these depend on many biotic and abiotic factors, such as season length, number of host species and number of parasitoid species, for example.

The cost to the parasitoid may be absent or low when viruses are recruited to disarm the host defences. On the other hand, the parasitoid must ensure that the viruses retain their symbiotic nature, which may involve mechanisms that are costly in terms of resources or energy. It is tempting to speculate that the VLPs are remnants of viruses resulting from natural selection for maintaining the benefits of the virus ability to interfere with the host immune response, but against the potentially harmful effects of the virus for the parasitoid. Information on the cost of resistance in parasitoids has been investigated in several systems. The use of selective breeding and molecular techniques is allowing detailed understanding of the mechanisms underlying the costs and benefits within these systems. Kaech et al. (2021) investigated differential gene expression of different *Hamiltonella defensa* strains, their host, the aphid *Aphis fabae* and its secondary endosymbiont *Buchnera aphidicola*. Using RNA-Seq analysis they were able to identify specific differences in gene expression in the host and *H. defensa* strains, but not in *B. aphidicola*, that underpin the costs/benefits of the different strains on their host. However, if we wish, for instance, to understand geographical patterns as described for *Asobara tabida*, it is essential that future research should focus on cost in parasitoids.

Research investigating the cost of resistance in the host, has mainly focused on *Drosophila melanogaster*. As pointed out by Kraaijeveld et al. (1998), the critical issue is whether there are negative effects of having an encapsulation

response when the chance of parasitism is low. Using selected *Drosophila melanogaster* lines, Kraaijeveld and Godfray (1997) were the first to show a trade-off between being resistant and another life-history trait. Under limited food conditions, the resistant genotypes were worse competitors than susceptible genotypes. No other life-history trait differences were found between the selected lines. Finding trade-offs is difficult in any system, hence the paucity of data on costs may not reflect the absence of costs, but rather the absence of carefully designed experiments. Again, experiments on costs and benefits of resistance under different ecological conditions are needed to further our understanding of the genetics of host–parasite interactions.

Other systems consider the effects of bacterial symbionts on the interaction between the host and an associated parasite, parasitoid or pathogen (e.g., Ulrich et al., 2021). One example that has been exploited to create a novel method of disease control, is via the infection of the mosquito *Aedes aegypti* with the endosymbiotic bacterium *Wolbachia pipientis* (Frentiu et al., 2010). Flores and O'Neill (2018) reviewed this exciting area and reported that it has been shown that *Wolbachia* infection can limit the transmission of a range of human pathogens by *A. aegypti*, including dengue, Zika and chikungunya viruses.

3.5.4 QTL Mapping

Principles

Variance analysis, as discussed above, describes QTs in statistical terms. It is not known which genes or which alleles contribute to the genetic variance component and these may be different even when the values of variances are very similar for different populations of the same species. However, the development of molecular markers and linkage maps of these markers have ushered in the exciting field of molecular quantitative genetics. It is now potentially possible to elucidate quantitative polygenic traits in terms of the complex genetics of co-evolving genes and specific mechanisms. Genes that underly QTs are called quantitative trait loci (QTL).

The principles underlying QTL mapping are extensions of standard mapping techniques of major genes (Falconer & Mackay, 1996; Lynch & Walsh, 1998; Fig. 3.6). They use the association between marker genes from a linkage map and the putative gene affecting the trait under consideration. However, as explained above, QTs are not described in discrete, qualitative classes, but in terms of phenotypic values, distributions and variances. Because the number of genes that underlie QTs can vary, the association between the marker and any of these genes may be difficult to detect, depending on the heritability of the trait, the genotypic values of the alleles and the environmental variance. The key feature, therefore, is linkage disequilibrium (LD) between the marker and the QTL, with different marker genotypes having different expected average phenotypic values, because QTL are linked to the markers (Lynch & Walsh, 1998). Although in principle a straightforward analysis, practically, QTL analysis involves sophisticated statistical techniques (see below).

Requirements

The two basic requirements are: (1) a linkage map of markers fulfilling the criteria as discussed above (Sect. 3.2.4) and (2) genetic variation for the QT for (closely related) species, strains, between populations or within populations. Of course, to acquire maximum LD in the progeny resulting from crosses between units that vary for the QT, the units will ideally be fixed for both alternate alleles at the QTL and at the marker loci. Furthermore, ideally, alleles that increase the QT should be in one unit, and alleles that decrease the QT should be in the other, although dispersion of beneficial alleles can be detected as transgressive segregation of trait values in the offspring. This is generally true for crosses between closely related species, and such species have been successfully used for QTL analysis (True et al., 1997). It is an exciting prospect to use crossable *Drosophila* species to map genes for parasitoid resistance. However, only a handful of such pairs of species is likely to exist that produce both viable and fertile offspring.

Therefore, divergent selection lines are often a good second bet to ensure optimal success for QTL mapping.

The parental strains in the crosses can be either still outbreeding or highly inbred so that they can be considered isogenic. For the reasons given above, the latter situation is preferred. Two possible crossing designs are most often used. For both, the parental lines are crossed to produce F_1 progeny. These then can be crossed either back to one or both of the parents or crossed *inter se* to produce F_2. Only in the double back-cross or F_2 design can additive effects of QTL be estimated in an unbiased manner when dominance effects are present (Falconer & Mackay, 1996). An F_2 design is more powerful than a back-cross design, because in the latter only the heterozygous effect of an additive allele is detected, which is half that of the homozygous effect in the F_2 design (Falconer & Mackay, 1996). However, back-crossing designs applied to both parental lines take away most of this problem and have distinct advantages of their own. Recall that the choice of any particular design is also dependent on the genetic (dominant or co-dominant) properties of the markers used. In addition, notice that both the linkage map and the QTL analysis can be performed in one and the same experiment.

Subsequently, the resulting progeny are scored for their marker genotype on all of the marker loci and the individuals are scored for their phenotypic value. If a difference is found between the average phenotype values when pooled by marker genotype classes, it can be inferred that a QTL is linked to the marker(s). This analysis was originally done using single marker analysis (Tanksley, 1993). This has one major drawback, namely the genotypic effect of the QTL is underestimated as a result of recombination between the marker and the QTL (underestimation by $(1 - 2c)$; Falconer & Mackay, 1996). This problem was circumvented in the method of interval mapping, in which the association between marker and phenotypic effect is considered using pairs of markers (Lander & Botstein, 1989). The choice of analytical procedure depends on the marker spacing (Tanksley, 1993). When the marker spacing is <15 cM, single marker and interval mapping give nearly identical results. For markers spaced >20 cM but <35 cM, interval mapping provides the maximal benefit. For marker spacing >35 cM, even interval mapping is inefficient and gives poor results. Again, with the advent of NGS-based genotyping systems, marker density is rarely a problem today. Interval mapping is explained below in further detail.

Interval Mapping

Consider two markers, M and N, with two alleles each, and a QTL, A, with two alleles, in between the markers (after Falconer & Mackay, 1996). The recombination frequencies are c_{MN} between M and N, c_{MA} between M and A, and c_{AN} between A and N. If complete interference is assumed, then (following basic requirement 1; see above), $c_{MN} = c_{MA} + c_{AN}$. The genotypes of the two parental lines are $M_1A_1N_1/M_1A_1N_1$ (with genotypic value a) and $M_2A_2N_2/M_2A_2N_2$ (with genotypic value $-a$), respectively. Hence, the F_1 progeny are $M_1A_1N_1/M_2A_2N_2$ (with genotypic value d). In this example, the F_1 is back-crossed with the $M_1A_1N_1/M_1A_1N_1$ parent. Table 3.3 shows all possible gametes and their frequencies based on the recombination values. Contrasting the four possible marker classes (double recombinations can be safely ignored if markers are closely spaced) provides an estimate of the QTL effect and its relative position between the two markers:

$$M_1N_1/M_1N_1 - M_1N_1/M_2N_2 = a - d \quad (3.19)$$

$$M_1N_1/M_1N_2 - M_1N_1/M_2N_1 = (ac_{AN} + dc_{MA})/c_{MN} - (ac_{MA} + dc_{AN})/c_{MN} \quad (3.20)$$

Equation 3.20 can be rearranged giving:

$$M_1N_1/M_1N_2 - M_1N_1/M_2N_1 = (a - d)(c_{AN} - c_{MA})/c_{MN} \quad (3.21)$$

Table 3.3 Expected gamete genotypes in a back-cross design, their frequency and contribution to back-cross progeny mean. Since it is the differences between the marker classes that are of interest rather than the extent to which each marker class contributes to the overall back-cross progeny mean, actual marker class means are also given (after Falconer & Mackay, 1996)

F_1 gamete type	Frequency	Genotypic value	Contribution to back-cross progeny mean	Actual marker class mean
$M_1A_1N_1$	$(1 - c_{MN})/2$	A	$M_1N_1/M_1N_1 = a(1 - c_{MN})/2$	a
$M_1A_1N_2$	$c_{AN}/2$	A	$M_1N_1/M_1N_2 = (ac_{AN} + dc_{MA})/2$	$(ac_{AN} + dc_{MA})/c_{MN}$
$M_1A_2N_2$	$c_{MA}/2$	D		
$M_2A_1N_1$	$c_{MA}/2$	A	$M_1N_1/M_2N_1 = (ac_{MA} + dc_{AN})/2$	$(ac_{MA} + dc_{AN})/c_{MN}$
$M_2A_2N_1$	$c_{AN}/2$	D		
$M_2A_2N_2$	$(1 - c_{MN})/2$	D	$M_1N_1/M_2N_2 = d(1 - c_{MN})/2$	d

Tests and Computer Packages

The marker class means will have associated variances, therefore statistical tests are required to decide whether the differences found between the marker classes are significant. There are broadly two approaches to significance testing: minimising the residual sum of squares in multiple regression, or maximising LOD scores.

Multiple Regression

In the multiple regression technique, the phenotypic value of each marker class is regressed on the unknown QTL parameters (a, d, and c values) (Haley & Knott, 1992). Starting with a marker interval (of known position and spacing), the recombination values between QTL and flanking markers can be iterated (in small steps) until $c$values are found that result in a significant regression and maximise the sum of squares of the fit (or minimise the residual error). The coefficients of the regression function are then the estimates of a and d. If the first interval that was considered does not yield a significant result, the next interval is considered and so on. We refer to Haley and Knott (1992) for a full description of the expression of the regression coefficients as a function of the QTL parameters in a F_2 design and to Kearsey and Pooni (1996) for practical guidance and examples.

Likelihood Analysis

Alternatively, a likelihood function is specified in terms of the observed data and the parameters to be estimated. Combinations of the parameters are then tried to find the maximum likelihood solution (usually in c steps of 0.01–0.05). This solution is then tested with the LOD ratio (Sect. 3.2.3) between the observed likelihood function and the likelihood function of no QTL segregating (Lander & Botstein, 1989). This log odds ratio is χ^2-distributed. Although the level of significance is usually taken as $\alpha = 0.05$ in statistical hypothesis testing, QTL analysis is carried out for n markers spread over the whole genome, hence the usual $\alpha = 0.05$ per test is clearly inappropriate, so corrections have to be made for multiple testing. The appropriate LOD threshold above which a QTL on a certain map position is judged significant depends on the size of the genome and the density of the markers (i.e., the number of tests performed). This threshold can be found by addressing the question: what is the chance of finding a QTL (i.e., LOD scores passing the threshold) when no QTL is segregating in the population? (Lander & Botstein, 1989). The threshold value differs according to whether the maps are dense or sparse, in other words, whether LOD scores can be considered independent events or not. For a

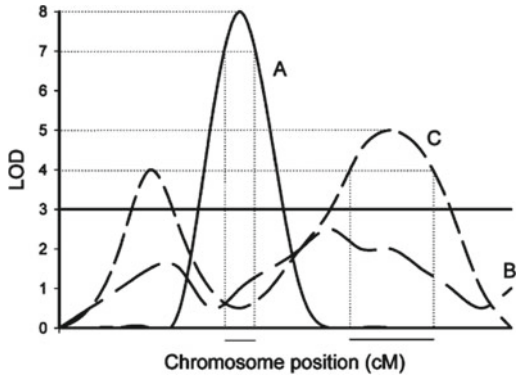


Fig. 3.20 Theoretical plot of LOD score against chromosome position. The solid horizontal line indicates the significance threshold value (see text for details). The three curved lines indicate three traits. For trait A, a QTL with strong effect is found; for trait B no indication is found for a QTL; for trait C, there is statistical support for two QTL. The precision of the map position of a QTL can be calculated as the one LOD support interval, which approximates 95% confidence intervals (Lynch & Walsh, 1998). This interval can be found by subtracting one LOD from the peak of the LOD plot and subsequently projecting onto the X-axis the points where the LOD − 1 line crosses the observed line (intervals indicated as horizontal lines on X-axis)

sparse map, the significance level per test is approximately α/n (Lander & Botstein, 1989; Lynch & Walsh, 1998). For dense maps, thresholds should be calculated depending on the map properties, and a general formula is given in Lander and Botstein (1989). Threshold values can also be calculated using permutation tests (Doerge & Churchill, 1996). The original data set is reshuffled many times using the observed phenotypic values but these values are now randomly assigned to the genotypes. The QTL test statistic is calculated for each of these synthetic data sets (while no association exists between marker and trait) to give a frequency distribution of test statistics. An empirical threshold value can be determined by finding the value of the test statistics belonging to the $(1 - \alpha)$ percentile. For more general discussions of significance thresholds for QTL interval mapping, see Doerge and Rebaï (1996) and Lynch and Walsh (1998). Figure 3.20 shows a schematic plot of the LOD score against chromosome position as often found in many QTL papers.

Composite Interval Mapping

If more than one QTL is segregating in the population, the marker class variance includes a genetic component due to these QTL unlinked to the interval. For instance, a QTL with a very large phenotypic effect may mask other QTL in the populations (Kearsey & Farquhar, 1998). By using additional markers as co-factors, these effects can be removed. This is known as composite interval mapping and it greatly increases the likelihood of detecting a QTL and also the precision of the estimated map position (Jansen & Stam, 1994; Zeng, 1994). In addition, there is a large body of literature on the statistical properties of testing for QTL, such as the analysis for multiple traits (Jiang & Zeng, 1995; Korol et al., 1995). An extensive review is provided by Lynch and Walsh (1998). Moreover, an analysis of the genetic architecture, including epistatic interactions between QTL, is possible using multiple interval mapping (e.g., Zeng et al., 1999).

The multiple regression technique was developed because the maximum likelihood approach is computationally demanding and could not originally be implemented in standard packages (Haley & Knott, 1992). The tests are related, however, and yield nearly identical results (Lynch & Walsh, 1998). So, multiple regression can be easily performed (see Doerge, 2002, for discussion of statistical and computational methodology). In addition, several computer packages are available including QTL cartographer (https://brcwebportal.cos.ncsu.edu/qtlcart/index.php) (Basten et al., 1997) and more recently MapQTL6 (https://www.kyazma.nl/index.php/MapQTL/). Both of the latter programmes can be recommended because they use a sequential procedure, starting with single marker analysis, followed by step-wise regression and finally interval mapping, composite interval mapping, and multiple interval mapping. MapQTL6 also offers a maximum likelihood approach to allow larger numbers of markers to be handled than is possible for regression-based approaches.

General Considerations and Problems

Mapping QTL for traits with high heritabilities is easier because they are less sensitive to environmental perturbations. However, even for such traits with high heritabilities, sometimes no QTL can be found, because detection depends on the heritability of a single QTL. Hence, with many genes the individual locus heritability can be very low. At first sight, the presence of QTL seems to falsify the polygenic nature of the QTs paradigm (see above). However, there is a strong bias towards QTL with a large effect being detected and such QTL are usually mapped with reasonable precision (Kearsey & Farquhar, 1998). Probably due to these properties, the number of QTL found generally equals the number of chromosomes (Kearsey & Farquhar, 1998). Tanksley (1993) and Kearsey and Farquhar (1998) provide more general observations on QTL and Mulligan et al. (2017) describe the GeneNetwork toolbox that allows analysis of increasingly large datasets.

The above illustrates the largest problem associated with QTL mapping: detection of QTL is possible but the confidence intervals around the map position are large (the one LOD support interval is large, Fig. 3.20 trait C). In connection with this, it is important to realise that a QTL does not necessarily mean a single locus: more than one gene may be present in the LOD peak found in the test statistics. Although developments in statistical analyses have greatly helped to abate the consequences of this problem, increasing the precision of phenotypic value estimates is necessary, through application of composite interval mapping-like approaches. The power of the test is dependent on the within-marker-class standard deviation (Falconer & Mackay, 1996). This standard deviation can be reduced by measuring more individuals (or rather genotypes) or by creating recombinant isogenic lines (RILs). In the latter, marker genotypes can be replicated many times and measured on several occasions, or in different environments, to study QTL–environment interactions. In *Drosophila melanogaster*, constructing RILs is straightforward using balancer chromosomes (Lindsley & Zimm, 1992). In addition, the haplodiploid system offers the unique possibility of creating (near) RILs (see above), but surprisingly they have not yet been exploited. Epistatic interactions are important in evolutionary theory (see above) but are difficult to detect in standard back-cross and F_2 designs due to lack of statistical power. It is not surprising, however, that epistatic interactions are documented in experiments using RILs because of the potential of repeated measurements on RILs (e.g., Long et al., 1995). The development of the multiple interval mapping techniques will facilitate the investigation of complex epistatic patterns.

This section will be concluded by briefly touching upon the use of QTL mapping in outbred populations. Mapping in such populations is considerably more difficult than in inbred populations, because the power is much lower. For instance, not all parents are informative, i.e., some are fixed for the marker, but not for the QTL or fixed for the QTL but not for the marker. In addition, in one family a marker allele may be associated with one allele of the QTL, but in another family the same marker allele is associated with another allele at the QTL. How to deal with these problems is discussed in Chap. 16 of Lynch and Walsh (1998).

Examples

The concept of QTL mapping was initiated from the field of plant and animal breeding, and it is therefore not surprising that most examples can be found there (e.g., Tanksley, 1993). It was realised that knowing the position of genes influencing economically important traits (such as milk yield in cows) offered the potential of crossing such genes into breeding populations (introgression). In such crosses the phenotype of the individuals would not necessarily have to be measured, but the markers closely associated with the gene of interest could be used as a flag. Molecular and genetic identification of the identified QTL also offered the potential for unravelling the genetic, developmental and physiological pathways in which the QTL was involved.

The recent adoption of NGS sequencing techniques now make it possible in many species

to detect historical recombination among "unrelated" genotypes. The principle being that they are historically related, even if very distantly, and only very close marker–marker or marker–trait linkages will still be intact. This approach, generally known as genome-wide association studies (GWAS) is very widely used in animal and plant systems but faces greater issues for implementation in insect systems (e.g., Pannebakker et al., 2020; Tamiru et al., 2020).

The genetic basis of wing-size differences between two *Nasonia* species has been investigated (Weston et al., 1999; Gadau et al., 2002). Males of *N. vitripennis* have short, rudimentary wings and are incapable of flying, whilst *N. giraulti* male wings are 2.4 times longer and the males can fly (although they have a low flight tendency). By curing the parental strains of their *Wolbachia* infections, the hybrid cross between the species resulted in viable and fertile F_1 females. *N. vitripennis* males of five eye-colour mutant strains were crossed to *N. giraulti* females. The mutants belonged to either of one of the five linkage groups (chromosomes) of *N. vitripennis*. The resulting hybrid females were subsequently allowed to lay eggs on hosts to produce male offspring. As mentioned above, all markers in haplodiploid males are informative and the males were scored for their wing size and eye-colour. The average size of the wing was compared between mutant colour eyes and wildtype-eyed males within one mutant line cross. When a significant difference was found between these groups, it was concluded that a gene influencing wing size was located on the same chromosome on which the eye-colour mutant was located. The frequency of eye-colour mutants with large wings and wildtype wasps with small wings was used to calculate how close the wing-size gene was to the eye mutant (as in Fig. 3.6). The findings from these crosses were corroborated in an introgression experiment. The major finding of this study is that a gene (or several tightly-linked genes) on chromosome IV explains 44% of the wing-size difference between the two species and is located 1.4 map units from the eye-colour mutant (Weston et al., 1999), whilst results of the F_2 hybrid analysis indicate there is another locus on linkage group 1. Gadau et al. (2002) were able to add further detail to the linkage analysis and identify epistatic effects. Since these early studies, further progress has been made (Lynch, 2015). The main region of large effect, *wing-size1* (*ws1*) (Weston et al., 1999) has been positionally cloned (Loehlin et al., 2010b) and is localised within a 13.5 Kb non-coding region, close to the sex-determining locus *doublesex*. A second locus *widerwing* (*wdw*) has been identified (Loehlin et al., 2010a). The linkage groups have been anchored to the five *N. vitripennis* chromosomes (Rütten et al., 2004) ; a high-density genotyping microarray using >20,000 *N. vitripennis* and *N. giraulti* markers has been created (Desjardins et al., 2013) and several genomes sequenced and annotated (Werren et al., 2010; Wang et al., 2020). The approach of Loehlin et al. (2010b) can be applied to a wide variety of phenotypic differences and, when combined with the increasing availability of genome sequences, provides a powerful technique to investigate this model parasitoid and other haplodiploid insect enemies. Further studies are likely to concentrate on the adaptive significance of the size differences and its evolution, together with the genetic analysis of differences in other traits, such as courtship, male aggression and host preferences. It is of interest whether these traits are determined by at least one gene with a large effect as well, or many genes with small effects.

Successful mapping of QTL has been reported for foraging behaviour in the honey bee, *Apis mellifera* (Hunt et al., 1995). Selection was applied on the amount of pollen stored in combs of honey bee colonies. The selected strains were then crossed, and QTL mapping for the selected trait was performed using a back-cross design and a RAPD marker-based map. Two QTL were identified, located on two different linkage groups. Interestingly, in a separate cross the QTL effects were demonstrated by the co-segregation of marker alleles (associated with the QTL) and individual worker behaviour. Moreover, the alleles of the two marker loci were found in different frequencies in the different foraging task

groups and the QTL appeared to affect both pollen load and nectar load.

Both the above examples illustrate that a QT does not necessarily have to be determined by many genes having a small effect. However, bear in mind the inherent bias towards genes with large effect in QTL analyses.

Concluding Remarks on QTL Mapping

Ensuring that most of the aforementioned problems are accommodated in the best possible way, QTL mapping (whether through controlled crosses or whole population approaches) is a potentially powerful and valuable tool for uncovering the genetic basis of QTs. Especially in the case where candidate genes are available, a better understanding can be developed for genetic variation of a trait in nature and why possible differences between populations or strains exist. Alternatively, in the absence of potential candidate genes in the interval where a QTL is mapped, the complete toolbox of molecular biology can be exploited to find the gene(s) underlying a QTL (Tanksley, 1993; Falconer & Mackay, 1996; Lynch & Walsh, 1998). This is, however, still a long way off for all but a few species.

3.6 Conclusion

The pace at which new technologies and analytical tools continue to develop is leading to a proliferation of new studies. These are providing greater insight and understanding of the detailed genetics that underlies the relationships and interactions between insect natural enemies, their hosts and the multitude of associated microbiota within their ecological networks and integral to their life histories. Should a species of interest not have been discussed here, a search of literature more recent than this chapter is recommended.

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Mating Behaviour

4

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4.1 Introduction

In sexually reproducing animals, reproduction entails insemination (the transfer of the male's sperm to the female) and fertilisation (the fusion of a sperm and an egg to create a diploid zygote). In many insect species, including predators and parasitoids, insemination and fertilisation are temporally separated, from minutes or hours to years (as in many eusocial insects). The term "mating behaviour" refers to the behavioural events surrounding insemination. These behaviours can serve to promote successful sperm transfer by the male and use by the female, but this is not always a given. First, apparently successful inseminations can fail to result in fertilisation for a number of reasons (see Greenway et al., 2015, for an overview). Importantly in the context of insect natural enemies, the fitness consequences of these 'cryptic' mating failures vary: for diploid predators, failing to produce fertilised eggs will result in complete reproductive failure whereas mating failures in hymenopteran parasitoids are less costly because, due to haplodiploidy, unmated females can still produce unfertilised eggs which develop as (haploid) males. Second, mating behaviours exhibited by many female insects serve to prevent insemination and fertilisation and allow females to exercise choice over which males sire their offspring. Alexander et al. (1997) divided mating behaviour into: pair formation, courtship, copulation, insemination, and the events immediately following insemination (including temporary pair maintenance). We discuss mating behaviour according to these divisions, with extensions to consider behaviours underlying female choice (before and after insemination) and post-copulatory male behaviours that have evolved in response to sperm competition (Parker, 1970a). Our discussion also includes components of mate competition and fertilisation. We take an approach that focuses on proximate mechanisms or causal relationships. The next chapter, on *Mating Systems* (Chap. 5), employs a complementary approach that considers ultimate causes (a 'functional' or 'adaptive' approach to understanding evolution).

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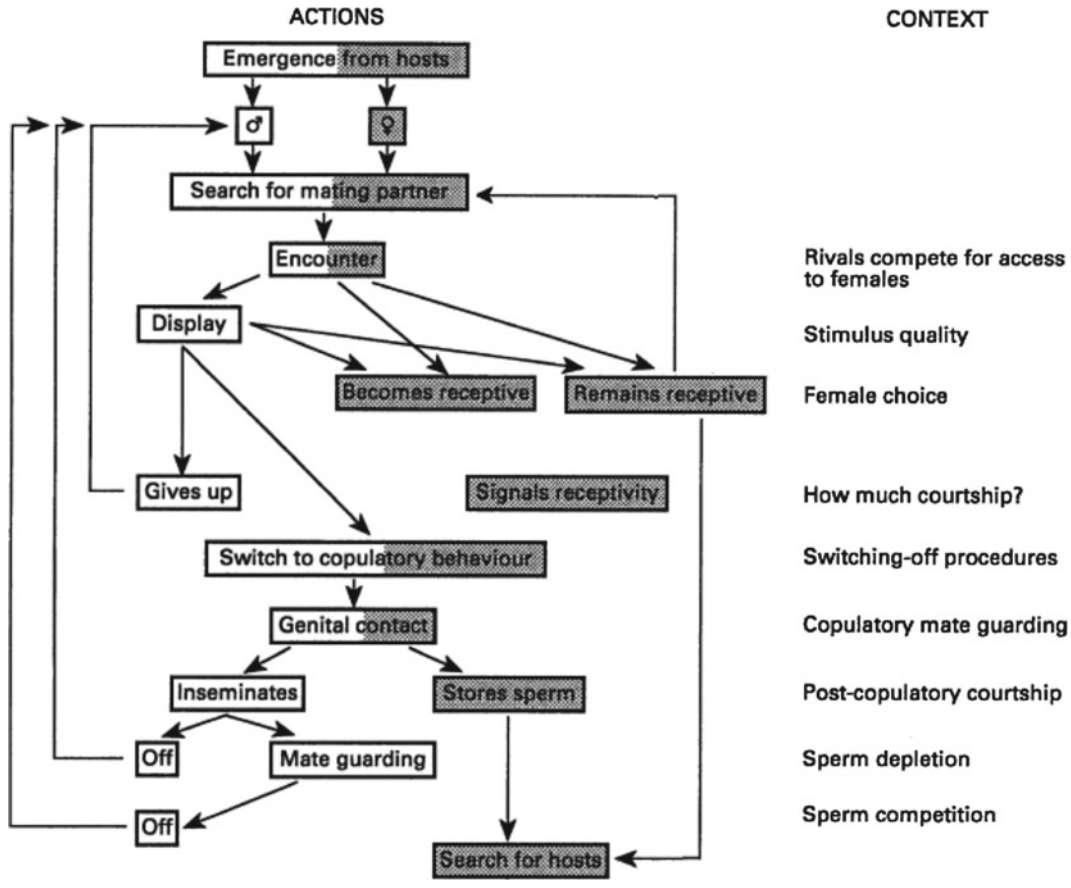

Fig. 4.1 Schematic representation of the course of events in a typical sequence of mating behaviour. Shaded and unshaded parts of boxes denote contribution by female and male, respectively

A typical sequence of mating behaviour, from the search for mates up to insemination and mate guarding is shown in Figs. 4.1, and 4.2 represents the behaviour of the parasitoid wasp *Cotesia rubecula* (Braconidae) as a specific example. However, the taxonomic and biological diversity of insect natural enemies is enormous. Consequently, our choice of examples is selective, concentrating mainly on van den Assem's studies of three species of chalcidoids: *Nasonia vitripennis* (Pteromalidae) and *Melittobia acasta* (Eulophidae), both parasitoids of cyclorrhaphous fly pupae, and *Lariophagus distinguendus* (Pteromalidae), a parasitoid of the larvae of grain weevils. More recent work on additional species has been incorporated into this edition, but these older examples remain very much relevant and still form the foundations of the chapter. This is because to mate successfully, and optimally, most parasitoids and predators need to solve similar problems; these are: where to find a partner, how to recognise it, how then to behave so that insemination, if desired in the case of females, will result and, in the case of males, what measures (if any) to take so as to uphold or enhance a sperm monopoly in fertilisation (i.e., how to counteract sperm competition).

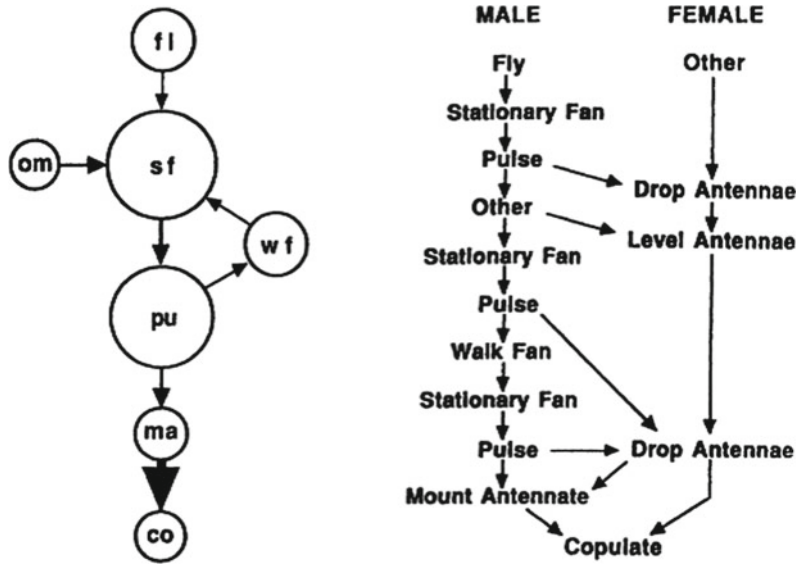

Fig. 4.2 A specific example: mating behaviour in *Cotesia rubecula* (Hymenoptera: Braconidae), a solitary endoparasitoid with a polygynous mating system and a typical sequence of behaviours during courtship and mating. However, mating behaviour is more complex when males compete directly for mating opportunities (Field & Keller, 1993a, Sects. 4.3.6.3 and 4.4.1, Figures 4.26 and 4.8). The left-hand flow diagram shows the sequence of male behaviour during successful courtship (using abbreviations defined in Table 4.2), the areas of circles indicate the relative total durations of each behavioural component and the widths of arrows show the extent to which the duration of each behaviour exceeds expectations (for details on how this was calculated, see Field and Keller 1993b). The right-hand flow diagram shows interactions between male and female in typical courtship. Females emit a sex pheromone and lower their antennae (when receptive). Males produce low-frequency acoustic emissions associated with wing movement (stationary fan) and 'pulses' of vibrational signals transmitted through the substrate (Sect. 4.3.4). 'Other' behaviours by males occur randomly and are followed by stationary fan. There are usually three repetitions of the stationary fan-pulse behavioural act in a successful sequence. From Field and Keller (1993b), with permission from Plenum Publishing Corporation

Table 4.1 A two-by-two summary of Tinbergen's four questions, after Bateson (2012)

	Current	Historical
Proximate	How does it work?	How did it develop?
Ultimate	What is it for?	How did it evolve?

4.1.1 The Hows and Whys of Investigating Natural Enemy Mating Behaviour

Mating behaviour can be studied from several points of view. Tinbergen (1963) considered four fundamental questions that can be asked of any behaviour (see Bateson & Laland, 2013, for a detailed review of Tinbergen's approach, and Table 4.1 for a summary):

1. How does it work? What is the proximate causation and organisation of the behaviour? This is the main approach taken in this chapter. Such studies might involve identifying which mechanisms underlie the production of behaviour and determining how these mechanisms operate, determining which factors (internal and external) release the behaviour, resulting in its manifestation, understanding how incoming information is processed, and how motor activities become

co-ordinated activities which can be observed as behavioural acts.
2. What is it for? What is the adaptive function (i.e., the ultimate causation) of the behaviour? This involves measuring the fitness consequences of behaviours, particularly in terms of reproductive success (this is the approach taken in Chap. 5).
3. How did it evolve over the history of the species? What are the steps by which behavioural patterns have changed over evolutionary time and have assumed their present form, and the directions any such developments have taken? We discuss this briefly at the end of this chapter and, in Chap. 5, we consider methods (e.g., phylogenetic ancestral state reconstructions) that can be used to investigate such changes in evolutionary time.
4. How does it develop over the lifetime of an individual? What is the ontogeny of the behaviour? This involves studying when a behaviour is performed, whether it is instinctive and is performed automatically at a certain life-history stage or whether some stimuli or learning are needed to trigger it.

There are several practical reasons for studying the mating behaviour of natural enemies:

1. Studies of mating behaviour can help to identify the attributes that make a given species an effective biological control agent. For instance, species that mate close to where they emerge (both spatially and temporally) and do not show strong inbreeding avoidance or mate choice might be more suitable for biocontrol as they may be more likely to remain in the vicinity of the release site such that regular subsequent releases are less necessary. In a similar vein, investigations of mating behaviour are likely to be invaluable additions to the predictions made by population models of host–parasitoid dynamics (Luck, 1990). Studying mating behaviour may be critical in predicting the success of biological control introductions and establishments (e.g., Hopper & Roush, 1993) as well as in developing efficient techniques for the mass rearing of insects that are required in large numbers at specific times; e.g., for field or greenhouse releases (Waage et al., 1985; Hall, 1993; Heinz, 1998). Understanding responses of males to female sex pheromones may be useful in monitoring parasitoid populations (Suckling et al., 2002). Mating behaviour among beneficial natural enemies may also be disrupted by exposure to synthetic pesticides, such that their capacity for biological pest control is diminished, and mating behaviour evaluations could therefore be usefully included in the screening and approval procedures for new insecticides (Benelli et al., 2014; Tappert et al., 2017).
2. Mating behaviour studies may also prove to be useful in the identification of a species to be introduced as a biological control agent (Gordh & DeBach, 1978). Given the high degree of host specificity of many natural enemy species, it is essential that biocontrol practitioners are certain about the taxonomic identity of the insects they intend to use. Moreover, practitioners must be certain that any species that is introduced cannot hybridise with natives (Hopper et al., 2005). Species-specific courtship displays often provide a strong barrier to hybridisation and can be used to detect morphologically (and even genetically) indistinguishable species (Danci et al., 2006; Sohani et al., 2012). Analyses of mating behaviour may be the easiest means of distinguishing between species because, in general, mating displays tend to be species characteristic which prevents costly cross-species matings (van den Assem & Povel, 1973; Matthews, 1975, see also Hunter et al., 1996; Kazmer et al., 1996; Heimpel et al., 1997; Heraty et al., 2007; Rhoades, 2015; Bredlau & Kester, 2019).
3. Studies of mating behaviour in insect natural enemies provide some of the clearest tests of several behavioural theories including many of those which fall under the umbrella of sexual selection (selection which results in differences in fitness through non-random success in competition over mates and their gametes; Shuker & Kvarnemo, 2021; see also

Andersson, 1994; Godfray, 1994; Eberhard, 1996; Boulton et al., 2015). Furthermore, parasitoid wasps are outstanding organisms with which to demonstrate the basic tenets of insect mating behaviour in the classroom (e.g., Barrass, 1976). Additionally, haplodiploidy has consequences for sexual conflict and sexual selection and so comparisons of hymenopteran parasitoids with predators and other diploid parasitoids provide a valuable opportunity to test how processes such as sexually antagonistic coevolution are affected when males do not have fathers (de la Filia et al., 2015).

4.2 General Methodology

4.2.1 Field Versus Laboratory Studies

Most detailed investigations are carried out in the laboratory because many parasitoids are very small, and observing and measuring their behaviour can require specialised equipment. Also, environmental conditions and some key characteristics (such as size or age) of insects used in experiments can be standardised under laboratory conditions. Important variables such as egg load (Sects. 2.3.4–2.3.6) or time between successive matings are difficult to measure and control in field experiments. Despite this, field observations are vital for understanding whether the mating behaviours observed in the laboratory reflect those seen in nature. Meta-analyses (Gates, 2002; Oliveira et al., 2021) are increasingly used to assess broader evolutionary patterns of mating behaviour across species, but these analyses have also demonstrated how methodological details can influence interpretation (Torres-Vila et al., 2004; Dougherty & Shuker, 2015). In order to fully understand the function and fitness consequences of any behaviour, controlled experimental work should be combined with field observations (Ewing, 1983). Field investigations such as Parker's (1970a, 1970b, 1970c, 1970d, 1971, 1978) work on dung-fly mate searching are valuable as they consider mating behaviour in a realistic ecological context and are less prone to experimental artifacts than laboratory studies. Parasitoid mating can in some cases be observed in detail under field conditions (Tagawa & Kitano, 1981; Takahashi & Sugai, 1982; Antolin & Strand, 1992; Field & Keller, 1993a; Fauvergue et al., 1999; Adams & Morse, 2014) but often it is difficult to distinguish between individuals. Unless behavioural studies are coupled with subsequent genetic analysis of parentage (Chap. 3), an assessment of an individual's success within a group is often impossible. Relatively large, and thus easily visible, wasps (e.g., in the families Sphecidae, Pompilidae or Tiphiidae) or dragonflies can be marked either with paint or tags glued to the dorsum (see Field et al., 2007, for an example in ammophiline digger wasps). Such marks can even be applied to small parasitoids (Driessen & Hemerik, 1992; Field & Keller, 1993a; see Hagler & Jackson, 2001, and Hagler, 2019, for reviews of marking techniques for insects). Fluorescent dyes and powders have been also used to monitor dispersal in the field (Corbett & Rosenheim, 1996; Rice et al., 2015). These techniques may prove useful in tracking individuals when studying mating behaviour, but this is yet to be validated in parasitoids. Naturally occurring phenotypic markers such as eye- or body-colour mutants, and resistance to insecticides, have also proven to be useful ways to investigate the behaviour of individuals (e.g., Ode et al., 1995; Baker et al., 1998). Transgenic lines and RNA interference (RNAi) can also be used to manipulate individual phenotypes (Santos et al., 2014; Walton et al., 2020) and their gametes (Marie-Orleach et al., 2014), and track mating behaviour and reproductive success. In cases where manipulated traits are purely for identification purposes (i.e., the effect of the trait itself is not being tested; as in Santos et al., 2014) care must be taken to ensure that marks or altered traits do not alter behaviour or reproductive success (i.e., Howard et al., 2004). Gene editing, knockdowns, and RNAi have been used in insect natural enemies to study their evolutionary ecology (Lynch, 2015; Dalla Benetta et al., 2021) and applications for biocontrol are much discussed (see Christiaens et al., 2020 and

references therein). These techniques also hold great potential for studying the mating behaviour and mating systems of natural enemies (Walton et al., 2020). We hope that, as this technology becomes more cost-effective and widely available, these techniques will be integrated into studies of natural enemy mating systems, helping to disentangle the ultimate causes and proximate underpinnings of the varied mating behaviours these species exhibit.

4.2.2 Culturing Insects

Long-term culturing may influence insect behaviour. In *Nasonia vitripennis*, for example, mated females become increasingly receptive to remating after multiple generations in laboratory culture (van den Assem & Jachmann, 1982; Burton-Chellew et al., 2007b). This change appears to be related to relaxed constraints on sperm use for adaptive sex allocation and direct fecundity benefits associated with mating multiply (Boulton & Shuker, 2015; Boulton et al., 2018a, 2019, Sect. 5.4.7), but the evolutionary significance of these findings outside of the laboratory remains to be elucidated.

Typically in *N. vitripennis* and many other parasitoids, female receptivity is 'switched off' after a single mating (Sect. 4.3.6), sometimes regardless of whether insemination has been successful or not (Boivin, 2013). Changes to this 'switching-off' mechanism seen in laboratory-adapted *N. vitripennis* are unlikely to be restricted to one species. Whilst care must be taken when interpreting data from long-term laboratory cultured populations, such laboratory artefacts can also shed light on how ecology can alter selection on mating behaviour and mating systems (Boulton et al., 2018a, 2019) and allow rates of evolutionary change to be quantified (Hoffmann & Ross, 2018). In other cases, laboratory adaptation has not led to apparent shifts in mating behaviour or capacity. Gordh and DeBach (1976) found no difference in sperm production and insemination potential between *Aphytis lingnanensis* (Aphelinidae) males from 25-year-old cultures and males from field collections. Similarly, Kazmer et al. (1996) argued that long-term culturing was not the cause of observed reproductive incompatibilities between strains of *Aphelinus asychis* (Aphelinidae).

In general, insect predators exhibit higher female mating rates both in the laboratory and in nature, and so increased female receptivity under laboratory adaptation may go unnoticed. Laboratory adaptation has been less well documented in predators compared to parasitoids, but selection under mass culture is ubiquitous across insect taxa (Hoffmann & Ross, 2018). There are likely many examples of laboratory adaptation in more cryptic traits, particularly those relating to post-copulatory sexual selection, in highly polyandrous insect predators, but these remain to be discovered. In cases where laboratory adaptation is undesirable and not under direct study, ways of circumventing its effects include: (1) keeping separate mass cultures that are periodically mixed (van de Zande et al., 2014), (2) maintaining (and testing) many inbred isofemale lines, or (3) periodically augmenting with field-collected material (Nunney, 2009). Another method is to keep part of the parasitoid stock quiescent (e.g., in cold conditions, Jucker et al., 2020) or in diapause for extended periods.

4.2.3 Standardising Insect Material

As is true of most behavioural experiments, studies of mating behaviour should use individuals that have been standardised with respect to factors such as age, prior mating experience, and body size that affect the willingness or ability to mate. If standardisation is not possible, these variables should be statistically controlled for when interpreting observations.

Age differences are an important source of variation in courtship behaviour in males (Barrass, 1960a; Tagawa et al., 1985; Cheng et al., 2003; McClure et al., 2007; Jiang et al., 2018; Akinyemi et al., 2021) and responses to courtship in females (Jiang et al., 2018). In many cases the age of individuals of one sex also influences the mating decisions of potential partners. For

instance, in predators (Jiang et al., 2018) and parasitoids (McClure et al., 2007) younger males exhibit more vigorous and less discriminate (Akinyemi et al., 2021) courtship displays and may be preferred as mates. In other cases, older males achieve greater mating success than their younger conspecifics (Adams & Morse, 2014), perhaps due to experience. More broadly, in many parasitoid species newly emerged females are unreceptive to mating attempts (Godfray, 1994). In *Habrobracon* (= *Bracon*) *hebetor* (Braconidae) both males and females are unreceptive to mating immediately after emergence, a behaviour that is thought to reduce inbreeding levels (in *H. hebetor* inbreeding carries a fitness cost due to the genetic mechanism of sex determination, Ode et al., 1995). Furthermore, in some species unmated adult females become unreceptive after a certain period of virginity (Starý, 1970; Godfray, 1994 and references therein). Clearly, it is important to know the age of individuals, and this can be determined by monitoring pupae for adult emergence and subsequently holding the adult until any non-receptive period has passed.

Prior mating experience may also influence receptivity towards mating attempts. Most female insects mate with multiple males, but this is not the case in the parasitoids where, in 70% of species assayed, females mate only once (e.g., Wilkes, 1966; Kitano, 1976; Ridley, 1993; Allen et al., 1994; Ode et al., 1995; Hirose et al., 1988; Baker et al., 1998; Ruther et al., 2000; Chevrier & Bressac, 2002; Boulton et al., 2015). Given that post-mating non-receptivity is common among parasitoids, it is critical to know the mating history of individual males and females that are to be used in mating studies. Male mated status can also have profound effects on the outcome of mate-choice trials as it can influence both male motivation (King & Fischer, 2010; King & Miller, 2018) and female preference (Ruther et al., 2009). Sperm depletion is a common problem in insects; synspermatogenic males require time to replenish their sperm reserves and prospermatogenic males will remain sperm depleted for life after several mating attempts (Sect. 4.4.4). In addition to sperm, in some species males require sufficient seminal fluid proteins, spermatophores or pheromones to successfully mate (Sect. 4.5.2). Depletion of any of these resources can influence the fitness of their mates and result in selection on female mating preferences for virgin males (Boulton & Shuker, 2015). Virgin males and females can be obtained by isolating pupae in separate vials and holding each adult until it is to be used in an experiment.

There is also evidence that body size can affect male and female mating behaviour and success. There are numerous examples where large males gain greater mating success than their smaller conspecifics (Assem et al., 1989; van den Antolin et al., 1995; Joyce et al., 2009; Blaul & Ruther, 2012; Macedo et al., 2013) but in other cases small males are at an advantage (Burton-Chellew et al., 2007c; Blaul & Ruther, 2012) or there is no size-dependent advantage (Cook et al., 1994; Cheng et al., 2003; Joyce et al., 2009). Larger females in most insect species generally gain a fecundity advantage over their conspecifics, as they have more resources available to invest in egg production (Honěk, 1993). But size can alter female mating behaviour in a number of ways. For instance, larger more fecund females can have higher sperm requirements and so may show greater propensity to remate. On the other hand, larger females can also reject mating partners with greater ease than smaller females, and so can be less likely to remate (Crespi, 1989). Additionally, size incompatibility can inhibit successful copulation, leading to assortative mating, as Hunter et al. (1996) showed to be the case in the aphelinid *Eretmocerus eremicus*. As such, it is important to control for size, or test for size-dependent polyandry, mate choice and assortative mating when studying mating behaviour (see also Gwynne & Simmons, 1990; Boulton & Shuker, 2015).

Despite the importance of standardisation, testing for effects of factors such as mating status, age and body size on the behaviour and decisions of males and females can demonstrate how sexual selection operates in predators and parasitoids. Viewing these results in the context of the mating system (Chap. 5), particularly in the light of typical field sex ratios and sex differences

in emergence time, can be very fruitful when interpreting the biological significance of mating preferences and behaviours in ecologically relevant contexts.

4.2.4 Handling Insects

Handling prior to experiments should be kept to a minimum or done in such a way that its effects are minimised or standardised. For example, wasps can easily be coaxed from one vial to another by using their positive phototactic responses. To transfer a wasp from a vial into an observation cell (see below), simply tap the vial base of the tube once or twice; some species of wasps will retract their legs and so fall out. In some species, males seem able to grip a surface more strongly than females (probably an adaptation to prevent being pushed away by rivals or by unwilling females). Another method which is suitable for larger, more robust species is to use a mouth aspirator to introduce wasps into the observation cell. For smaller species, wasps can be captured between the soft hairs of a camel-hair brush and moved by gently manipulating the brush. Handling can also be facilitated by anaesthetizing with CO_2 or briefly cooling in the fridge to slow movement. Both CO_2 and chilling have been shown to influence mating behaviour in *Drosophila* (Barron, 2000, see also Nicolas & Sillans, 1989) and so if these techniques are used it is important to standardise across treatments, and where applicable test the extent to which these manipulations influence natural mating behaviour. Nevertheless, low temperatures slow down behaviour, so enabling accurate analysis of complex movements. For example, rates of display in *N. vitripennis* can be halved if the temperature is set at 13 °C, below which displays do not occur (Jachmann and van den Assem, 1996). However, methods must be validated with appropriate temperature controls as prolonged cold could alter behaviour qualitatively as well as quantitatively and can change species-typical outcomes of sperm competition, including patterns of sperm precedence (Giraldo-Perez et al., 2016).

4.2.5 Controlling Ambient Temperature

Maintenance of appropriate ambient temperatures (and other environmental parameters such as humidity and light) is important in order to collect behavioural data that are ecologically relevant and occur in nature. Insect metabolism and activity are strongly temperature dependent (see below), and ambient temperature is known to influence mating success in several insects (e.g., Larsson & Kustvall, 1990; Patton & Krebs, 2001; Roux et al., 2010).

Artificial illumination used for microscope observations may influence behaviour and mate choice if temperature and lighting conditions under which normal mating takes place are significantly altered (Botha et al., 2017). Fibre-optic illumination provides good lighting without also heating the observation area. Alternatively, reflective tags can be used to observe and identify individuals in low light-intensity arenas (Rayner et al., 2020). Further measures can be taken to maintain constant temperature, such as fitting brass observation cells with Peltier units (illustrated in van den Assem, 1996), which transport heat through a semiconductor by a reversible current. Temperature is set and monitored by a control unit and is accurate to 0.2 °C. Temperatures down to around −10 °C can be obtained, but extra measures are required to dissipate heat. Extreme low temperatures can be used to immobilise wasps (Sect. 4.2.4) and so allow precise experimental manipulations (e.g., sealing of mouthparts, Sect. 4.3.4).

4.2.6 Observing and Recording Behaviour

Observing the mating behaviour of large parasitoids (e.g., Ichneumonidae) and many predators is possible with the naked eye, sometimes within larger chambers or cages allowing for expression of more realistic behaviours, such as mating swarms, than is possible in smaller arenas. However, most parasitoids and many predators are only a few millimetres long and

magnification is needed (10× is usually sufficient). At high magnification, reduction both in the field of vision and the depth of focus severely constrains the ability to observe many behavioural details. Consequently, parasitoids and small predators generally need to be confined within small arenas for observations and recordings with microscope and/or video camera. Suitable observation cells can be made, for example, by cutting 7 mm-thick slices from transparent plexiglass tubing (internal diameter 25 mm). This diameter fills the visual field of dissection microscopes at 10× magnification. Cells can be capped with cover slips (45 × 45 mm) and placed on a strip of paper to allow slight repositioning of the entire cell during observations (illustrated in van den Assem, 1996). Automation of the camera's horizontal plane of movement to track mate-searching parasitoids can be helpful in overcoming the limited field of view of most video cameras (Fauvergue et al., 1998).

Confining insects in small spaces can have unintended, undesirable consequences. For example, chemical substances can accumulate and affect the further mating behaviour. As such, re-using the same vials without very thorough cleaning is inadvisable. In *N. vitripennis*, females will sometimes spontaneously exhibit receptivity (opening their genital pore; Sect. 4.3.6) without courtship when exposed to males that are courting other females (van den Assem et al., 1980). This can lead to mating failure due to a state of 'pseudovirginity' (van den Assem, 1969) if females are not receptive to mating more than once. The effects of artificial confinement on mating vary across species. For instance, *N. vitripennis* do not mate within the host puparium, while others (including the congener *N. giraulti*) do (Leonard & Boake, 2006; Trienens et al., 2021). As a result of within-host mating, volatile olfactory cues are likely to be inefficient and so selection should favour alternative modes of sexual communication (Böttinger & Stökl, 2020). Furthermore, the order in which males and females are introduced in a chamber (e.g., introducing a male into a vial containing a female or *vice versa*, or introducing a male and a female into a fresh vial simultaneously) is likely to have different effects on mating behaviour. In some parasitoids (e.g., *N. vitripennis*), males apply substances (functioning as chemical signals, Sect. 4.3.4) to the walls of vials while, in others, males find females via pheromone trails (see Boulton et al., 2015 for a summary). Factors such as these should be taken into account when designing mate-choice studies, particularly when testing mate choice or investigating barriers to between-species matings.

At its simplest, recording basic behavioural sequences requires only a notepad and a stopwatch if the timing of separate events, or intervals between them, is of interest. However, many acts are very complex and rapid, and sequences of acts must be recorded in some way if they are to be successfully described. Video recording is often used because behavioural sequences can be repeatedly examined 'frame by frame' or analysed using machine learning (see below) until all details have been noted (Benelli et al., 2020). Such records are indispensable for making precise descriptions of movements, and particularly for measuring temporal relationships between behavioural components. Once a movement has been seen in detail, it is much easier to recognise at normal speed and subsequently to notice any differences in performance. Video records are very useful when comparing related species (Sect. 4.6). There are certain disadvantages to the use of video recording. For example, it is not always possible to view the entire field of action continuously, which becomes a problem when interactions between individuals are studied. Additionally, processing many hours of video manually can be very time-consuming, particularly when the video must be observed at low speeds to pick up all salient details.

The alternative to the use of hand-written or video records is machine encoding. Historically, the standard event recorder was a mechanical device comprising a collection of keys with corresponding pens, ink, and paper (e.g., the 20-pen *Esterline Angus* recorder described in van den Assem, 1996, and used by him for many years). Electronic event recorders are now commonly used, extremely sophisticated and well

integrated with other research equipment, including software and hardware systems, such as The Observer XT (Noldus, 1991; Tourtellot, 1992; Davis, 1993; Visser, 1993) or JWatcher (which is free to download; Blumstein & Daniel, 2007) for live observation and Ethovision XT (Noldus et al., 2000, 2001) for video tracking and automated behavioural observations (see http://www.noldus.com for updates). Whereas Ethovision XT is more effective for tracking the spatial positions of individuals than for observing fine details of behaviour, Observer XT and JWatcher are event-logging software packages that allow an observer to code behaviours of interest, quantify them, and conduct basic analyses. BORIS (Behavioural Observation Research Interactive Software; Friard & Gamba, 2016) is free and open-source software that incorporates an event recorder and can be used to code live behaviour as well as video and audio recordings, and carry out various analyses. The cost-effectiveness and user friendliness of tablets and other hand-held devices such as smartphones which are compatible with such software as well as more general applications for data entry has significantly streamlined data entry, processing and analysis in recent years. The Observer software has been used in several studies of parasitoid mating behaviour (Field & Keller, 1993b; Allen et al., 1994; Table 4.2), as has the more recently developed Observer XT (Mair et al., 2017; Tappert et al., 2017; Jatsch & Ruther, 2021), and Mowles et al. (2013) used JWatcher. More recently machine learning (where computer algorithms are trained to characterise/discriminate data without being explicitly programmed) has emerged as a useful way to analyse behavioural data when datasets are too large and complex to manually process, score and analyse (Valletta et al., 2017).

Finally, the males of many parasitoid wasp species produce acoustic stimuli during courtship (Sect. 4.3.4). Such acoustic emissions can be recorded in a number of ways. Before the widespread availability of digital microphones and sound recorders, recordings were made via the substratum using devices that act as transducers; these included electro-dynamic microphones (van den Assem & Putters, 1980) and gramophone styli (Ichikawa, 1976). Condenser microphones allow recordings to be made from the air, rather than the substratum (i.e., Danci et al., 2010). Sivinski and Webb (1989) give many technical details on how to record acoustic signals of the braconid wasp *Diachasmimorpha longicaudata*. Experiments can be carried out in which recorded male signals are played back into the substrate (e.g., a plant) or surroundings and the female's responses observed (Danci et al., 2010; Villagra et al., 2011). For instance, Danci et al. (2010) and Villagra et al. (2011) have shown that playbacks of male wing fanning alter female behaviour compared to control playbacks of white noise in both *Glypanteles flavicoxis* and *Aphidius ervi*. Additionally, digital sound recordings can be converted into sonograms and other visual representations of sound and analysed to explore the effects of natural variation in sound parameters such as frequency and amplitude on behaviours such as mate choice (see Sect. 4.3.4 and Fig. 4.9 for an example). This latter approach has not had such widespread uptake for studies of insect natural enemies as other taxonomic groups, but the sensitivity of widely available hardware means that this is now possible. The International Bioacoustics Society (IBAC, https://www.ibac.info/links?category=09) provides a useful summary of this hardware, and available software for analysing acoustic data.

4.2.7 Describing Behaviour—Ethograms

To describe a behavioural sequence, an ethogram is required. Ethograms are a library of, often sequential, behavioural events or 'acts' which are given a code and defined in an objective, mutually exclusive way. Behaviours are not interpretative or descriptive of perceived function (Qualitative Behaviour Assessment is a related approach which does utilise perceptions but requires a greater degree of cognitive similarly between the human observer and the animal subjects; e.g., Cellabos et al., 2021). Ethograms

Table 4.2 Components of *Cotesia rubecula* (Hymenoptera: Braconidae) courtship behaviour identified during observations prior to the main experimental observations and used to configure event recording software (Sect. 4.2.6); the abbreviations in parentheses are used in Fig. 4.2. From Field and Keller (1993b), with permission from Plenum Publishing Corporation

Behaviour	Description
Female	
Drop-antennae	Head tilted forward, antennae held together and pressed down on substratum in front of body
Level-antennae	Antennae held parallel to substratum, intermediate between raised and drop-antennae
Walk	Moving forward, usually alternately touching the antennae to the substratum
Groom	Any cleaning of the body, including stroking the face and antennae with legs, or cleaning the body with mouthparts
Fly	Any airborne activity
Other	Any other behaviour (predominantly 'point', in which the wasp faced upwind with anterior portion of body raised and antennae raised and spread, which occurred at commencement of observation)
Male	
Fly (fl)	Any airborne activity
Stationary-fan (sf)	Standing with antennae raised and spread, wings raised and fluttering
Walk-fan (wf)	Walking with wings raised and fluttering
Pulse (pu)	Stationary (occasionally walking) wing fanning interspersed with rapid pulsing movements of the abdomen and flexing of legs. Wings pushed down towards horizontal with each individual pulsing movement
Mount-antennae (ma)	Male placing fore and mid tarsi on dorsal surface of female gaster, rapidly antennating female head, and curling gaster underneath body to make genital contact
Groom	As for female
Walk-antennae	Palpating the substrate with both antennae while walking
Stationary-antennae	Palpating the substrate with both antennae while stationary
Wave-antennae	Stationary and waving antennae just above the female's body without contacting her
Other (om)	Any other behaviour, including pointing
Female and male	
Copulate (co)	Male establishing genital contact and leaning upper part of body backward with fore legs only on female gaster, wings raised and antennae spread and vertical

allow behaviours to be scored consistently by multiple observers (Sect. 4.2.8), including those who have not witnessed the behaviour first hand before (Martin & Bateson, 1996; Bateson & Martin, 2021). An ethogram of the courtship behaviour of the parasitoid wasp *Cotesia rubecula* is provided in Table 4.2 as an example (Field & Keller, 1993b; see also Fig. 4.2). The records produced should reflect the dynamics of behaviour as accurately as possible. However, there are usually so many aspects of even a simple behavioural sequence that it is impossible to represent them all in one record. Usually, several body components (e.g., limbs, antennae, head, abdomen) take part in a posture or a behavioural sequence such as a display. One approach is to record the sequence of events for each part of the body separately. One can subsequently examine how the movements of the sets of components are co-ordinated and determine whether any temporal relationships are recognisable. While motor patterns of movements may be relatively invariable, the intensity and orientation of these movements as well as the timing of successive

events may vary greatly. Furthermore, events may occur in rapid succession or concurrently and these problems are compounded when studying the behaviour of interacting pairs of animals, in which case two observers may be required (e.g., Field & Keller, 1993b; Sect. 4.2.8). Once the behavioural sequence has been characterised, analytical tools such as the R package *sequence* can greatly streamline quantification and analysis (Pierre, 2020).

4.2.8 Measuring Behaviour

Two commonly used methods used to measure behaviour are 'focal' and 'scan' sampling. Focal sampling involves watching an individual and recording all relevant behaviours (Sect. 4.2.7) performed and so provides durations and frequencies of everything an individual does over a certain period. Scan sampling involves observing an individual at various time intervals and recording its behaviour. Scan sampling has the benefit of allowing an observer to collect data from multiple individuals at once, but at the expense of the higher resolution and detail, including information about behavioural sequences, which can be provided by focal sampling. When mating behaviour is being observed, the duration of courtship and copulation will often guide the choice of method. In most parasitoids courtship and copulation can be very brief and key elements could be missed with scan sampling (although see Boulton & Shuker, 2016, for an example of scan sampling to record harassment and courtship in *N. vitripennis*). In other insects, including some predators, courtship and copulation can last several hours, and scans can be carried out infrequently without missing any important behaviours (e.g., Brown et al., 2012).

Once an ethogram has been developed (Sect. 4.2.7) and a sampling method has been decided on, it is important to assess the quality of the behavioural data collected. Martin and Bateson (1996) and Bateson and Martin (2021) give thorough reviews of why and how to assess the quality of behavioural data which we advise anyone to read prior to conducting a study on mating behaviour. Perhaps the most important component of quality for any behavioural study is consistency. This can be achieved by compiling a specific and detailed ethogram (Sect. 4.2.7) which can be validated by repeatedly observing the same behaviour and scoring consistency. When multiple observers are recording behaviour, it is important to ensure that they are scoring a given behaviour in the same way. Between-observer reliability measures the extent to which a single or different observers score behaviours consistently and get the same results. Even when there is only one observer it is advisable to test within-observer reliability to ensure that the observer is scoring the same things consistently over time. Video recording can be useful to allow reliability to be easily assessed and there are numerous metrics that can be calculated to test both within- and between-observer reliability (see Martin & Bateson, 1996, and Bateson & Martin, 2021, for specific details).

4.3 Pair Formation and Courtship

4.3.1 Searching for Mates

Male and female parasitoids developing on the same host, or patch of hosts, with high levels of inbreeding (Sect. 5.4.2) may not need to search extensively for mates. At one extreme, in *Nasonia giraulti* and other gregarious species, mating occurs within the host puparium prior to emergence (within-host mating) so that mate searching after emergence is unnecessary (Leonard & Boake, 2006; Trienens et al., 2021). In other gregarious or quasi-gregarious (solitary development on hosts with a clumped distribution) species, including *N. vitripennis*, newly emerged males remain on or near the host, and begin courting as soon as a female emerges. Males can locate females within a host puparium, in some cases using chemical cues, and characteristically position themselves on the puparium surface (King et al., 1969; Steiner et al., 2005; Danci et al., 2011). In some cases, males chew through host casings to mate with females before they emerge, or even 'assist' females with emergence

(Gordh & Debach, 1976; Suzuki & Hiehata, 1985). In other gregarious species (e.g., *Melittobia* spp.; van den Assem, 1976a; Gonzalez et al., 1985; Abe et al., 2005; Innocent et al., 2011), females approach males. This is likely because the sex ratio is so female biased (proportion of offspring that are males: 0.02–0.05, Abe et al., 2021; Iritani et al., 2021) that the availability of males limits female fitness (van den Assem et al., 1982a). The sex ratio is strongly female biased in *Melittobia* because the first emerging male typically kills all the other males, either as pupae or as eclosing adults (most males are siblings in this gregarious species; Abe et al., 2003, see also Abe et al., 2021; Iritani et al., 2021). As such, the first emerging male obtains most or all matings, after committing fratricide. Mated females are no longer attracted to males and become positively phototactic and move away from the host.

In solitary species whose hosts do not occur in masses, mate searching by one or both sexes is necessary. In the case of parasitoids, mate searching by males is more common than by females. This is likely because the OSR (operational sex ratio: the ratio of males *versus* females ready to mate at a given time; Emlen & Oring, 1977; Kvarnemo & Ahnesjö, 2002) is typically male biased, and male fitness is more limited by mating opportunities than female fitness (Bateman, 1948). This is particularly true in parasitoids, where haplodiploidy means that females can still gain some fitness as virgins by laying unfertilised (male) eggs (Sect. 5.3.3). Mate searching is likely to be concentrated in areas where the probability of mate finding is highest; i.e., sites where females emerge, feed, or oviposit (Fig. 4.3, Sect. 5.3.4). When sites are in short supply or are spatially clumped males may monopolise these sites, often exhibiting territorial behaviour. In many insect species, males aggregate in areas which do not contain resources (leks and swarms) which females visit to obtain matings. Leks and swarms appear to be less common in predatory insects and parasitoids (Shelly & Whittier, 1997) but Antolin and Strand (1992) have demonstrated the existence of leks on the surface of grain piles in *Habrobracon hebetor*.

Where mating does not occur on emergence or in territories, other means of mate finding are required. Chemical, acoustic, tactile, and visual stimuli may be used by individuals of either sex to find mates (e.g., Cole, 1970; Vinson, 1978; Eller et al., 1984; Kamano et al., 1989; Field & Keller, 1993a, 1993b, 1994; Fauvergue et al., 1995, 1998, 1999; McNiel & Brodeur, 1995; Pompanon et al., 1997; Steiner et al., 2005; Danci et al., 2011; Astaraki et al., 2019).

Chemical signals for mate attraction are usually volatile and provide long-distance information about the signaller's general, but not precise, location. However, virgin females of some species lay down trails of sex pheromones, which may or may not be volatile compounds: e.g., *Aphelinus asychis*, a solitary parasitoid of aphids (Fauvergue et al., 1995, 1998; Kazmer et al., 1996), *Aphytis melinus* (Aphelinidae) a parasitoid of scale insects (Bernal & Luck, 2007), *Trichogramma brassicae* (Trichogrammatidae), a gregarious parasitoid of crambid moth eggs (Pompanon et al., 1997; Fauvergue et al., 1998), *Metaphycus luteolus* (Encyrtidae), a facultatively gregarious parasitoid of scale insects (Kapranas et al., 2013), *Urolepis rufipes* (Pteromalide), a solitary parasitoid of fly pupae (Würf et al., 2020), and *Ascogaster reticulatus* (Braconidae),

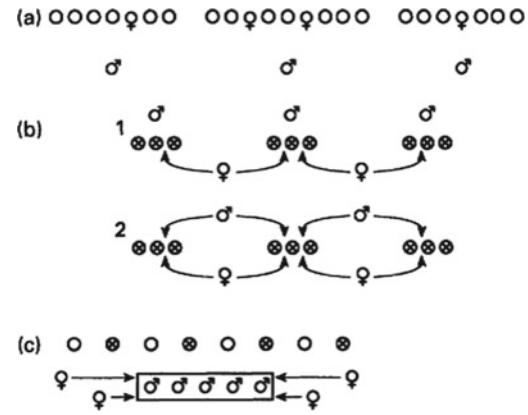

Fig. 4.3 Schematic representation of different mate-searching strategies: **a** males monopolise sites where females emerge; **b1** males monopolise sites where females oviposit or search for food; **b2** males move continually between such sites; **c** sites cannot be monopolised, males aggregate and may signal in concert

an egg-larval parasitoid of tortricid moths (Kamano et al., 1989). Males typically respond to an encounter with the pheromone by intensively searching in or near the marked area. Because the pheromone effectiveness dissipates within 24 h, it provides a reasonably precise cue to the location of virgin females. Fauvergue et al. (1998) compared the response to trails and patches of pheromones deposited on the substrate by males of *A. asychis* and *T. brassicae*. They predicted that male *A. asychis*, in which both sexes usually emerge in different localities, would follow pheromone trails to find females and that male *T. brassicae*, in which both sexes emerge from the same locality, would remain on pheromone patches in order to encounter females when they emerge. Contrary to expectation, male *A. asychis* do not follow trails any more than male *T. brassicae* and male *T. brassicae* do not stay longer in pheromone patches than male *A. asychis*. In fact, the response to pheromone is quite similar in these species. One possible explanation is that there is more non-local mating in *T. brassicae* than expected and that males of this species, like male *A. asychis*, commonly mate with females that have emerged in other localities, as observed in other species of *Trichogramma* (Kazmer & Luck, 1991; see also Schumm et al., 2020, and Sect. 5.4.4).

Acoustic signals produced by, or associated with, wing vibration can give precise information on the signaller's locality. Small insects need to produce very high-frequency sounds to communicate through air over anything but very short distances (Michelsen et al., 1982; Michelsen, 1983). In *Glyptapanteles flavicoxis* male wing vibrations appear to induce females to take short flights, and the associated flight sounds attract males to the female's location for mating (Danci et al., 2011). In structurally diverse environments such as vegetation, high frequency sounds are unsuitable for long-range communication due to scatter, reflection, and interference, and so substrate-borne vibrations tend to be used. Some parasitoids produce such signals (e.g., Field & Keller, 1993b; Danci et al., 2010; Villagra et al., 2011), but evidence that these function in the same way as long-range communication signals is lacking.

Passive visual signals are probably effective over longer distances only during daylight hours and where there is little cover (e.g., at the surface of bodies of water or in open spaces, many dragonflies exhibit brilliant sexually dimorphic colours or conspicuous markings, which function in mutual recognition; e.g., Grether, 1996). However, bioluminescent visual signals are particularly effective at night. Fireflies emit species-characteristic coded flashes (Lloyd, 1971) and have specially adapted euconic compound eyes to perceive them (Chapman, 2013). All signalling incurs predation risks. For example, the females of some firefly species attract heterospecific males by mimicking the female of that species, but then feed on, rather than mate with, the hapless male (Lloyd, 1975). Perception of visual cues over greater distances requires large eyes of sufficient acuity: in many swarming insects where being the first male to spot a female can mean the difference between reproductive success or failure, there is considerable sexual size dimorphism in eye size (e.g., honeybees, empidid flies, e.g., Downes, 1970). In contrast to larger predatory species, parasitoid wasps such as chalcidoids, proctotrupoids and cynipoids are too small to perceive long-range visual signals: only short-range (probably a few centimetres) visual communication is possible. This might contribute to the absence of elaborate and colourful sexual signals in the parasitoids (Boulton et al., 2015).

4.3.2 Mate Detection and Response

In general, in the parasitoids males initially detect females by olfaction (females also respond to olfactory cues produced by males; Sect. 4.3.4). On detecting a female, males increase their rate of antennal movement, vibrate their wings, and commence walking rapidly. Similar reactions are obtained when males are presented either with homogenates of females on filter paper (Obara & Kitano, 1974; Yoshida, 1978; Steiner et al., 2006; Nichols et al., 2010; Kapranas et al., 2013; Stökl et al., 2014) or with dismembered parts of females (Vinson, 1978; Field & Keller, 1994) or

dead females (King & Dickenson, 2008; King & Miller, 2018; Würf et al., 2020; Jungwirth et al., 2021). *Anagrus* (Mymaridae) males will attempt to copulate with a fine brush that has been wiped on virgin females (Waloff & Jervis, 1987). Solvent-extracted residues from females (e.g., acetone, diethyl ether, pentane, hexane, methylene chloride; see Jatsch & Ruther, 2021, for a discussion of solvent use in parasitoid studies) induce males to perform the initial stages of display or even, as observed in *Pteromalus puparum*, produce a complete display and copulate *in vacuo* (van den Assem, 1996). Intact females taken from the solvent and dried fail to produce a reaction, whereas dead females immersed in water and subsequently dried do produce a reaction. Clearly, males need not 'recognise' the object in its entirety in order to behave towards it as though it were a female: they react to chemical substances that release mating behaviour (van den Assem, 1996; see also Mair et al., 2017; Würf et al., 2020). Similar responses are found in insects that live in environments where visual communication is impaired.

Attempts have been made to locate the source of the courtship-initiating pheromone in a number of parasitoids and more recently advances in chemical ecology have led to the identification and synthesis of fractions of pheromones responsible for triggering behavioural responses (Ruther, 2013, provides a review; see also Mair et al., 2017; Würf et al., 2020). For instance, in *Urolepis rufipes* female cuticular hydrocarbons (CHCs) function as contact sex pheromones that elicit courtship (wing fanning and copulation attempts) by males. Only the non-polar fraction of whole-body extracts from females elicited responses by males; fractions containing polar lipids did not (Würf et al., 2020). Further, removal of n-alkanes from female-derived CHCs, which are a mixture of n-alkanes and methyl-branched alkanes, showed that the methyl-branched fraction alone did not elicit courtship responses, nor did synthetically produced n-alkanes. Two methylalkanes have higher relative abundance in the CHC profiles of females than in males but addition of these to male-derived CHCs did not elicit courtship by males. The methyl-branched alkanes and the n-alkanes probably act synergistically within the overall chemical profile to elicit courtship behaviour (Würf et al., 2020).

Pheromones appear to emanate from within widely different parts of the body. In several *Cotesia* species, a pair of glands in the female's reproductive system produce a sex pheromone (Tagawa, 1977, 1983; Weseloh, 1980; see also Field & Keller, 1994). In *C. glomerata*, however, the pheromone emanates from a gastral segment (Obara & Kitano, 1974). In the braconid *Diaeretiella rapae*, extracts from female gasters initiate courtship whereas extracts from other body parts do not (Askari & Alishah, 1979), while in the eulophid *Aprostocetus hagenowii* courtship is stimulated by extracts from the female thorax, but not from either the head or the gaster (Takahashi & Sugai, 1982). Using female pupae of different ages instead of adults, Yoshida (1978) found that pupae of the pteromalid *Anisopteromalus calandrae* secrete the courtship-eliciting pheromone, with a peak value around the red-eye stage (with older pupae there was a sharp decline in the effect). Full effects reappeared in adult females following emergence (a similar pattern has been reported for several Lepidoptera and Coleoptera). A simple experiment for distinguishing between chemical and visual stimuli in male courtship is illustrated in Fig. 4.4. Visual stimuli may work over very short distances. *Nasonia vitripennis* males will follow a small black object that is moved to and from behind glass a short distance away (Barrass, 1960b).

4.3.3 Orientation During Courtship

Courting chalcidoid males either mount the female or remain alongside or facing the female (van den Assem, 1976b; Figs. 4.5 and 4.6). Confrontation with a real female is often unnecessary for male courtship and mating behaviour to be elicited. Dummy females can be used to investigate which cues males respond to in courtship. A crude dummy made of cork, plywood or similar material may suffice, provided it carries the correct chemical cues

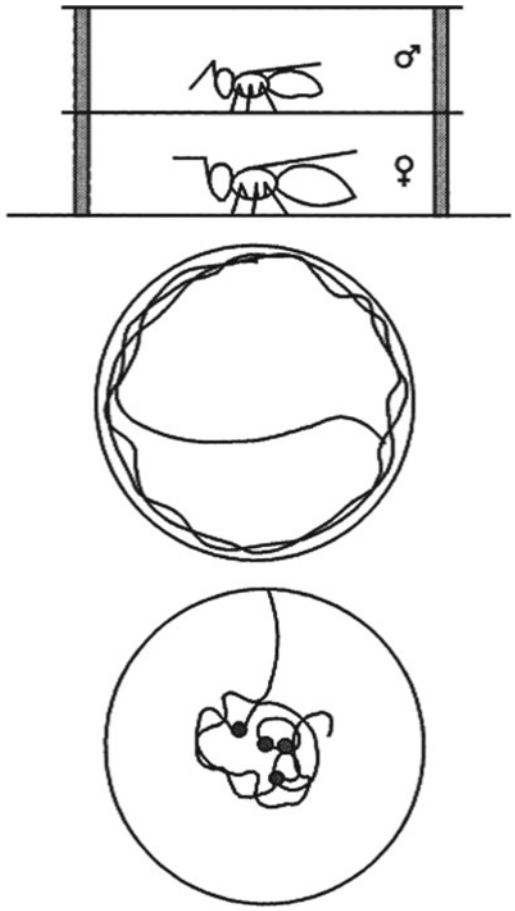

Fig. 4.4 Experimental set-up for demonstrating the effect of chemical cues in the parasitoid *Anisopteromalus calandrae*. Two glass rings (20 mm diameter, 4 mm high) are separated either by a thin glass cover or a millepore filter. With the glass there is no obvious reaction on the part of the male, which is walking along the wall of the cell, with the filter he restricts searching to the area with pores, together with antennations and wing vibrations (dots) (*source* Yoshida, 1978)

(obtained from females that have been immersed and agitated in a volatile solvent, Sect. 4.3.2). Within limits, its actual size is unimportant. However, males presented with a very crude dummy will rarely proceed beyond moving in an agitated manner while vibrating their wings, and will soon dismount (Yoshida, 1978; Yoshida & Hidaka, 1979; Tagawa & Hidaka, 1982). Yoshida and Hidaka (1979) tested whether males orient themselves in response to gravity. Dummies were attached to styrofoam at various angles: courtship position was determined by the female's posture relative to the substratum and not by gravity.

Male display position is mainly stereotyped but may differ between taxa. *Nasonia vitripennis* males mount the female and place the fore-tarsi on the female's head (placement of the tarsi of the other legs depends on the relative size of the female). A stereotyped position requires specific stimuli for a correct orientation, and these can be identified using dummies on which different real body parts can be glued, in various positions (Fig. 4.7). It is possible to determine what cues males use to arrive at and remain in the correct position and perform courtship behaviour. In *N. vitripennis*, the position of the wings provides directional cues (to front or rear), and an object (antennae, ovipositor, or an unnatural object) protruding at the end of a dummy seems necessary for display to be initiated (Fig. 4.8). Chemical stimuli are also involved. A display directed at an ovipositor lasts for only a short period, after which the male turns around and moves to the front of the dummy. A male that courts a dummy comprising a female's body and a male's head will display for a shorter period than on a dummy that is comprised entirely of female body parts (J. van den Assem, pers. comm.). Similar dummies have been used to investigate the behaviour of other parasitoid species (*Cotesia glomerata* and *Anisopteromalus calandrae*; Kitano, 1975; Yoshida & Hidaka, 1979).

4.3.4 Stimulus Production by Males

4.3.4.1 Introduction

Once a male parasitoid has assumed the correct orientation relative to the female, and the female has become immobile (presumably in response to the male's behaviour), the male will commence courtship. In some parasitoids, courtship displays are visually inconspicuous, whereas in others they are striking. According to Burk (1981), differences are likely to be correlated with the predominant mating system (Sect. 5.4). Displays are likely to be relatively simple in species when males defend territories containing resources that

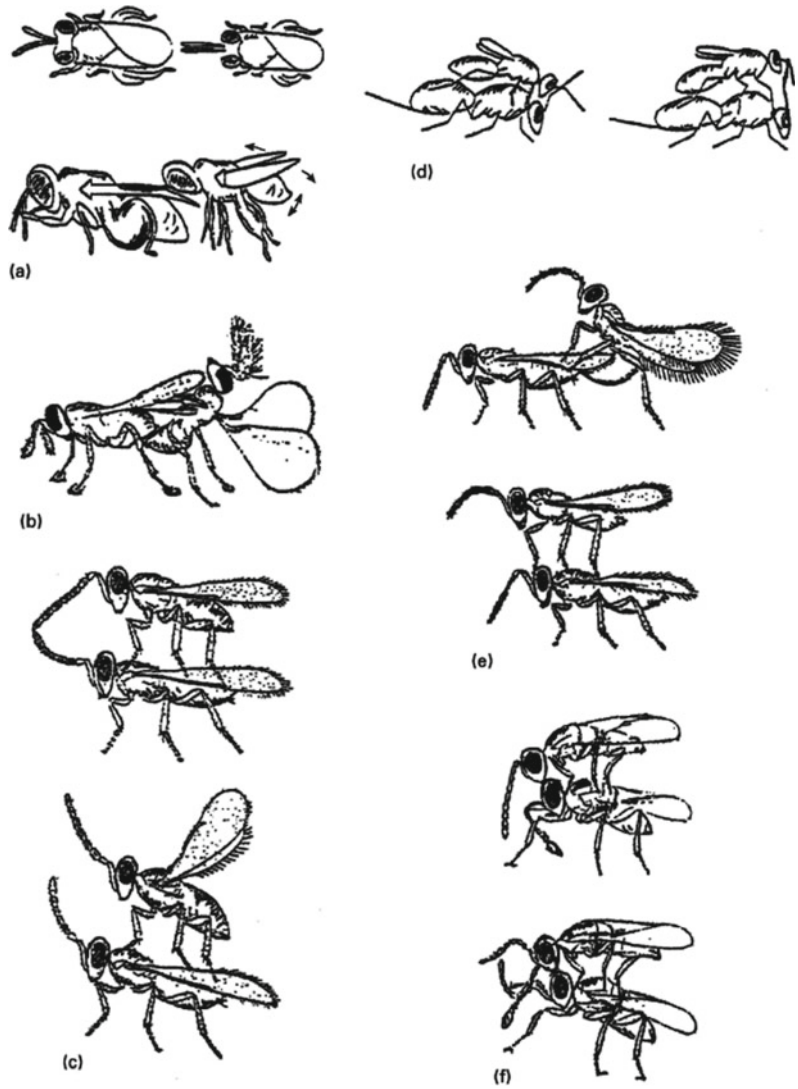

Fig. 4.5 Various courtship and mating postures of parasitoid wasps: **a** *Brachymeria intermedia* (Chalcididae), male following female, and male performing courtship movements (wing pressing, and buzzing) (*source* Leonard & Ringo, 1978); **b** *T. evanescens* (Trichogrammatidae) (*source* Hase, 1925); **c** *Encarsia partenopea* (Aphelinidae), phases of post-copulatory courtship (*source* Viggiani & Battaglia, 1983); **d** *Monodontomerus obscurus* (Torymidae), courting couple, male in low and in elevated position (*source* Goodpasture, 1975); **e** *Archenomus longiclava* (Aphelinidae), mating, and post-copulatory courtship (*source* Viggiani & Battaglia, 1983); **f** *Amitus vesuvianus* (Platygasteridae), phases of pre-copulatory display (*source* Viggiani & Battaglia, 1983)

females require (Sect. 4.3.1). Males generally do not display but attempt to copulate with any nearby female. Signalling is more complex in species where mate encounter rates are low or where females exhibit strong mate choice. Signalling is most extreme in situations where mating takes place within groups of competing males. However, these generalisations do not appear to hold for parasitoid wasps. Courtship in swarming species is not conspicuously complex (Nadel, 1987; Sivinski & Webb, 1989), whereas courtship in other species with very female-biased sex ratios, mating at the natal patch and even within-host mating often involves many behavioural, acoustic, and chemical elements (van den Assem et al., 1982a; Boulton et al., 2015). In the section below we elaborate on these components and we go on to consider female responses to these components in Sect. 4.3.6.

4.3.4.2 Acoustic Stimuli

The males of many parasitoid wasp species vibrate their wings, both upon approaching the female and during courtship *sensu stricto*. *Nasonia vitripennis* males produce acoustic pulses of a constant quality throughout a display and the pulses coincide with wing vibrations (Fig. 4.9). However, the wings themselves are

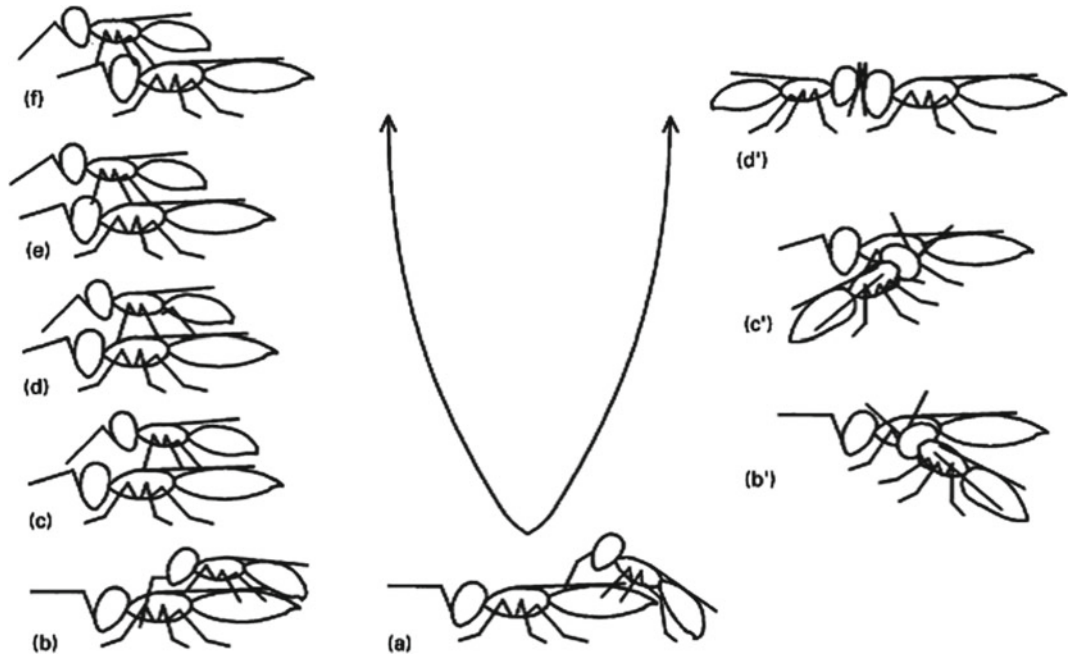

Fig. 4.6 Phylogenetic transformation series for the male's courtship position in Chalcidoidea using arbitrarily chosen examples (where no references are given the information derives from J. van den Assem's observations). The plesiomorphic courtship position is similar to the mating position in: **a** *T. evanescens* (Trichogrammatidae) and *Choetospila elegans* (Pteromalidae). In the left-hand branch the male mounts on the female, and his position moves gradually to the front; **b** *Spalangia cameroni* (Pteromalidae); **c** *Asaphes vulgaris* (Pteromalidae), *Sympiesis sericeicornis* (Eulophidae), *Aceratoneuromyia granularis* (Eulophidae); **d** *Pachycrepoideus vindemmiae* (Pteromalidae), *Vrestovia fidenas* (Pteromalidae); **e** *Nasonia vitripennis* (Pteromalidae), *Hobbya stenonota* (Pteromalidae), *Eupelmus spongipartus* (Eupelmidae), *Tetrastichus sesamiae* (Eulophidae), *Systole albipennis* (Eurytomidae); **f** *Anagyrus pseudococci* (Encyrtidae). In the right-hand branch, the male courts on the substrate; **b′** *Achrysocharoides* species (Eulophidae) (Bryan, 1980); **c′** *Pediobius* species (Eulophidae), *Tachinaephagus zelandicus* (Encyrtidae); **d′** *Microterys ferrugineus* (Encyrtidae) (Parker & Thompson, 1925). There is probably a third direction of development: males remaining at the rear but having peculiarly elongated antennae that reach out to the front (van den Assem, 1986)

not the source of the acoustic emissions because immobilising them, altering their surfaces, or removing them does not result in noticeable signal alteration. Similarly, males of the chalcidid wasp *Brachymeria intermedia* can be completely 'muted' by applying a tiny drop of superglue to the thoracic dorsum between the wing bases, preventing the thorax from acting as a resonator (Leonard & Ringo, 1978).

The pattern of acoustic emissions produced during courtship is species characteristic (Fig. 4.9), suggesting a display function which may serve in intraspecific mate choice (Benelli et al., 2013) and /or avoidance of heterospecific matings (Rungrojawanich & Walter, 2000; Bredlau & Kester, 2019). To investigate whether acoustic wing fanning displays function to induce female receptivity, the courtship efficiency (the amount of displays necessary to induce receptivity in virgin females) and mating success (the proportion of mated females producing progeny of mixed sex) of normal males, mute males and males with wings removed can be assessed (van den Assem & Putters, 1980). In *N. vitripennis* there is no difference between these three treatments; i.e., wing vibrations appear to play no role in courtship. Young mute males are able to induce receptivity within the same period as normal males and have similar mating success. However, females mounted by a mute male give

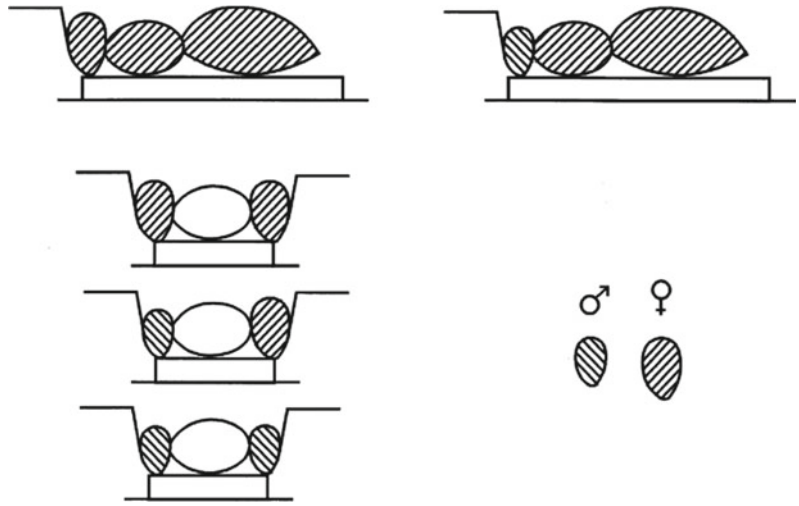

Fig. 4.7 Examples of dummies that comprise male and female parts

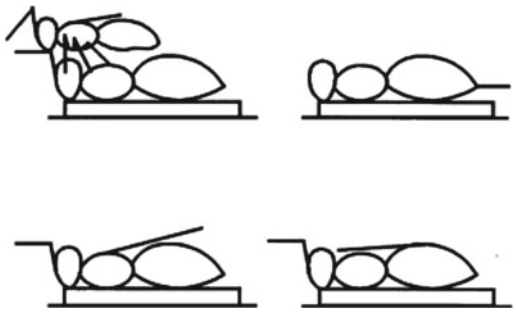

Fig. 4.8 Examples of dummies used to determine what cues males use to orient themselves on females, remain in position and elicit display behaviour

a 'startle response', as if they were mounted without 'advance warning'. Older males are apparently more successful at courting females if they produce sound (van den Assem & Putters, 1980). Mute, old males were presented daily with a sequence of virgin females. *Nasonia vitripennis* recordings and 'white noise' were alternately broadcast at a barely audible level. Displays were more successful when accompanied by courtship sounds than by white noise, indicating that stimuli are normally airborne (i.e., sounds) and are not substrate-borne. According to Barrass (1960a), old *N. vitripennis* males vibrate their wings less than young males and are less successful in courtship due to the deteriorating production of courtship 'sounds'. However, a deterioration in pheromone production, and/or the 'wafting' of that pheromone towards the female by the wing beating could also account for this observation (Ruther et al., 2009). Adding the acoustic emissions of *Mesopolobus mediterraneus* to the display of old, mute *N. vitripennis* males increased male success (van den Assem & Putters, 1980), suggesting that, in this case, emissions do not function in species recognition. However, the pitch of the *M. mediterraneus* emissions is similar to that of the *N. vitripennis* emissions (although the temporal pattern is different), so pitch may be an important cue.

In *Cotesia rubecula,* wing fanning by males produces both low-frequency airborne sounds and substrate-borne vibrations but it appears that only the latter can induce female receptivity. Field and Keller (1993a) observed males courting females that had settled on cabbage leaves. When males were on leaves adjacent to those bearing females, female receptivity was never induced, but the same males were normally successful in inducing receptivity when placed onto the same leaves as the females. Wing fanning is seen across the genus *Cotesia* and the vibrations that it produces may be involved in species recognition. Bredlau and Kester (2019) show that males from different species (including

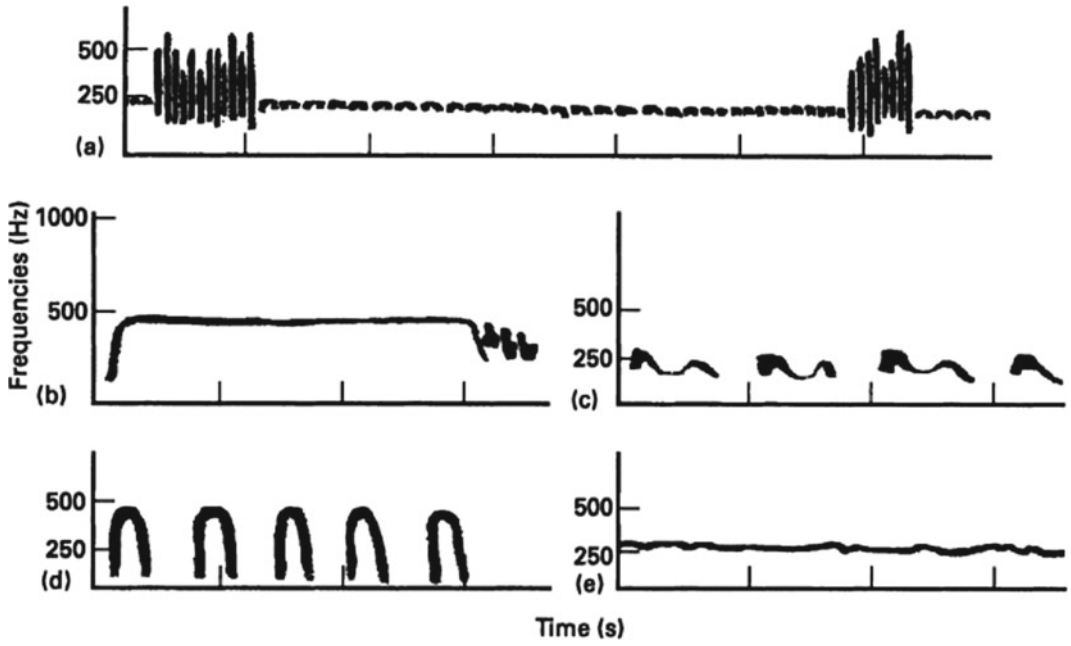

Fig. 4.9 Sonagram tracings of the courtship sounds of: **a** *Nesolynx ales* **b** *Baryscapus daira*, **c** *Tetrastichus sesamiae* (all Eulophidae), **d** *Nasonia vitripennis*, **e** *Anisopteromalus calandrae* (both Pteromalidae)

cryptic species) produce songs that vary in pulse duration and frequency. While the songs produced by males are clearly different, it is yet to be determined how females respond to conspecific *versus* heterospecific acoustic stimuli.

4.3.4.3 Chemical Stimuli

Chemical stimuli play an important role in short-distance communication between males and females (Sect. 4.3.2; Ruther, 2013). It is usually easy to establish whether chemical stimuli are involved in display and to determine their origin. Simple experiments with *N. vitripennis* have demonstrated their importance in inducing female receptivity (van den Assem et al., 1980; Mair & Ruther, 2019; Fig. 4.10). The onset of receptivity coincides with head nodding, a conspicuous display component, by the male (Sect. 4.3.6). As the head moves up the mouthparts are extruded, and they are retracted when the head moves down. Mouthpart extrusion coincides with the release of an oral pheromone which is transferred to the female antenna during courtship (Mair & Ruther, 2019; Fig. 4.10: 3). If males are manipulated so that head nodding and mouthpart extrusion are prevented (both separately and in combination; Fig. 4.11) the former males were able to elicit female receptivity while the latter were not (van den Assem et al., 1980). However, if air from a vial containing courting, normal, untreated males and females was released over the females, they immediately became receptive. Air taken from empty vials or vials containing only males or only females was ineffective. Sealing a male's mouthparts appears to prevent the release of a pheromone that coincides with mouthpart extrusion. Other pteromalids also extrude their mouthparts during courtship and this probably serves a similar function in eliciting female receptivity across the subfamily. It is likely that these aphrodisiac pheromones originate in the male mandibular gland (Mikó & Deans, 2014). The source of the pheromone could be investigated by dissecting out the glands, agitating them in a solvent such as hexane and carrying out a behavioural assay, although the wasps' small size may be prohibitive. A solvent extract might also be used to

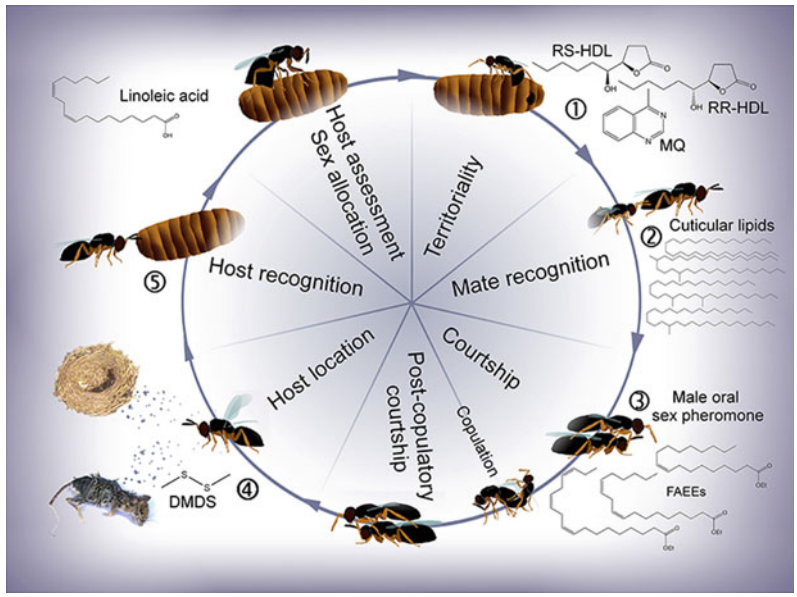

Fig. 4.10 Life cycle of *Nasonia* demonstrating stages at which different semiochemicals are used. (1) Males apply a sex pheromone to and around the host pupa which attracts and arrests females emerging from the host. (2) Males recognise females based on their cuticular hydrocarbons (CHCs, *N. vitripennis*) or culticular lipids (CLs = cuticular hydrocarbons + polar lipids, *N. giraulti*). (3) During courtship sex pheromones are emitted from the male mouthparts and transferred to the female antennae which is involved in inducing female receptivity and allows females to discriminate between con- and heterospecific courting males. (4) Females find new hosts using olfactory cues. (5) Chemical stimuli are also likely to be involved in host recognition, host assessment and sex allocation. RR-HDL, (4R,5R)-5-hydroxy-4-decanolide; RS-HDL, (4R,5S)-5-hydroxy-4-decanolide; MQ, 4-methylquinazoline; FAEEs, fatty acid ethyl esters; DMDS, dimethyldisulphide. Reproduced from Mair and Ruther (2019), *Frontiers in Ecology and Evolution* under the terms of the Creative Commons Attribution Licence (CC BY)

study, by electroantennography, the receptors involved (the latter are probably located on the females' antennae), but again the small size of most parasitoid wasps is likely to generate practical problems. Contact pheromones present on the cuticle are probably very widespread. Because of their species-characteristic properties they play a role in 'recognition' of mating partners. In some cases, cuticular hydrocarbons (CHCs, Blomquist & Bagnères, 2010; Würf et al., 2020; Fig. 4.10: 2) may be sex specific in terms of their CHC profile proportions. In the encyrtid *Tachinaephagus zealandicus*, males respond to the CHC profiles of freeze-killed females, but not freeze-killed males, despite both sexes possessing the same set of CHCs (Jungwirth et al., 2021). Once a display is underway, a constant input of stimuli originating from contact pheromones is necessary to keep the display going.

Although the substances eliciting female receptivity that are excreted during mouthpart extrusion are yet to be identified, Ruther and Hammerl (2014) have shown that three fatty acid esters in the mouthpart pheromone also initiate a behavioural switch in females. After exposure to these compounds (even without experiencing behavioural courtship) females are no longer attracted to the male abdominal sex pheromone (Fig. 4.10: 1) and switch to host finding (Fig. 4.10: 4) rather than mate seeking.

One method of simultaneously studying pheromone release and associated behaviour is to use time-course profiling of volatile release

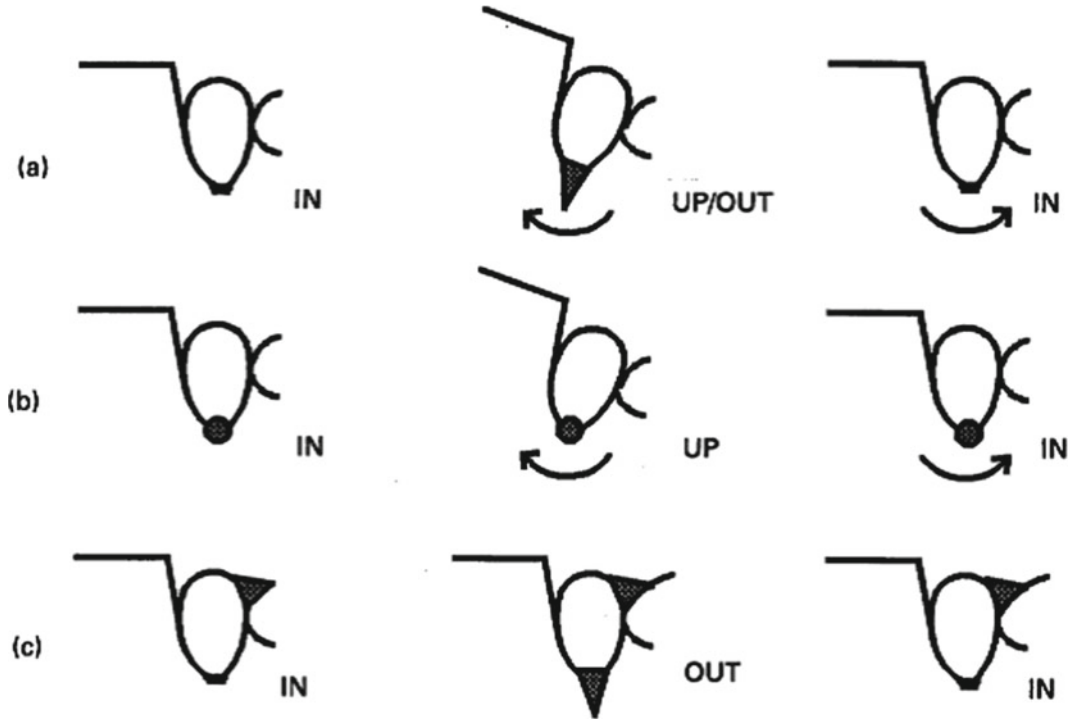

Fig. 4.11 Head nodding and mouthpart extrusion in *Nasonia vitripennis* males: **a** normal; **b** mouthparts sealed with superglue; **c** head movement prevented using superglue. Glue was applied to males immobilised by low (4 °C) temperature and hardened by adding a small drop of water

alongside observations or video recordings of behaviour. In this technique, originally developed by flavour chemists, insects are placed in an observation chamber which is connected to a mass spectrometer and volatile chemicals emitted are analysed directly in the gas phase (Taylor et al., 1995; Linforth et al., 1996; Linforth & Taylor, 1998; Harvey et al., 2000; Goubault et al., 2006, 2008; Goubault & Hardy, 2007). The method also provides an (inaccurate) measure of the amount of volatile(s) released. Mowles et al. (2013) have used this technique, employing atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS), to demonstrate that *Spalangia endius* females actively release a pheromone component during courtship and mating, releasing greater quantities in the later stages of mating and after having mated once (Fig. 4.12). Males took longer to initiate wing fanning in response to higher concentrations of the pheromone. This suggests that females actively secrete anti-aphrodisiac pheromones when they have already mated as a signal of non-receptivity to males. Kilpinen et al. (2012) used the same approach, based on proton-transfer reaction mass spectrometry (PTR-MS) rather than APCI-MS, to study bed bug mating behaviour.

4.3.4.4 Visual and Tactile Stimuli

The displays of many species of wasp involve leg movements, consisting of drumming, tapping, kicking, and swinging. All may be used in tactile stimulation of the females. Males of some species have conspicuously decorated legs, for example those of *Mesopolobus* (Pteromalidae), bear-coloured fringes or tufts which are moved in front of the female's compound eyes during courtship, suggesting a visual function (van den Assem, 1974). In the eulophid *Nesolynx albiclavus*, the male flexes his middle legs during a display. The tibiae are inclined steeply inwards

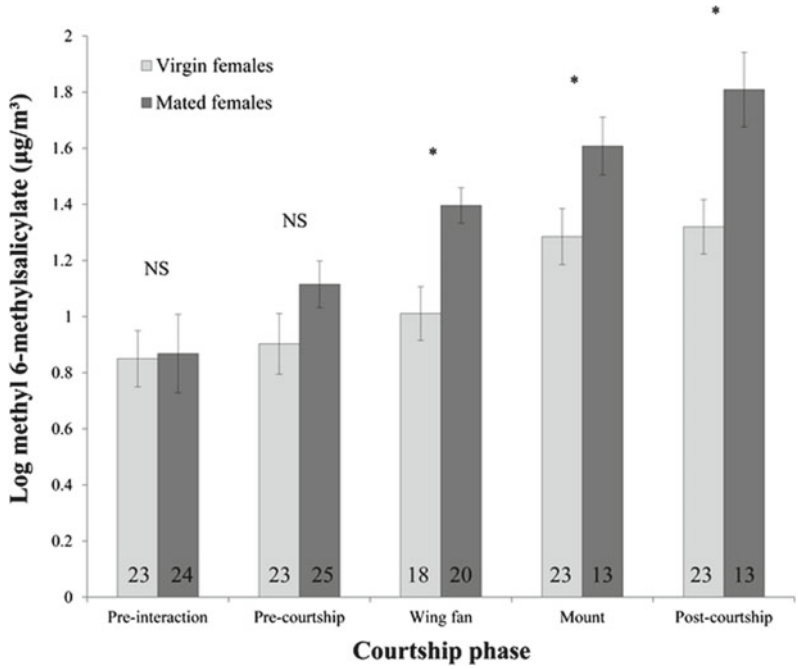

Fig. 4.12 Release of a sex pheromone (methyl-6-methylsalicylate) by females of *Spalangia endius* during courtship. Greater quantities of the pheromone are released in the later stages of courtship and by previously mated females. Error bars show ±1 standard error. Asterisks indicate significant differences within phases. As not all wasps progressed to each stage of courtship, numbers indicate sample sizes. Reproduced from Mowles et al. (2013), *PLoS ONE* (doi: 10.1371/journal.pone.0082010.g002) under the terms of the Creative Commons Attribution Licence (CC BY)

Fig. 4.13 Distal part of tibia and tarsi of the middle leg of a male *Nesolynx glossinae*, showing a structure that probably provides specific stimuli during courtship. From Graham (1989), reproduced by permission of Gem Publishing Co.

and the tarsi steeply outwards (an unusual posture), and the male drums on the female's thorax (van den Assem et al., 1982b). The tibial spur of the male's middle legs bears a basal triangular expansion from which long setae radiate, and the basitarsus bears remarkably long setae (Graham, 1989; Fig. 4.13). The posture of the legs is such that these structures will make contact with the female during drumming.

One method that has been commonly used in the past is to ablate structures that appear to have a tactile function (such as setae or legs) or cover/alter structures that appear to provide visual cues and measure the courtship success of altered males compared to normal ones (Uetz et al., 1996). By suppressing the expression of genes involved in producing mating stimuli, RNAi allows for more sophisticated manipulations of mating cues (and investigation of the genes that underlie them). It has been used to study how sex pheromones alter behaviour in insects, and how sexual ornaments develop (Gotoh et al., 2014; Walton et al., 2020). Knocking down/out traits using RNAi could also prove useful in assessing the effect of other cues

(visual, tactile, and auditory) on mating behaviour, but we know of no studies that have used manual manipulation (or RNAi) to alter stimuli associated with the mating behaviour of insect natural enemies.

4.3.5 Male Investment in Courtship

On meeting a conspecific female, males are usually ready to court immediately and thus to make an investment of time, energy, and materials, but how much should a male invest? A thorough quantification of a male's time, and perhaps energy, investment would require separation of the courtship display into its components (Sect. 4.2.7). These can be quantified in two ways: short-lasting events that can be tallied to provide a total number of occurrences, and longer-lasting events whose duration can be measured. For general accounts of how to quantify behaviour, see Sect. 4.2.8 and Martin and Bateson (1996) and Bateson and Martin (2021).

In terms of overall temporal patterns, displays can be classified as either cyclical or finite (van den Assem, 1975). Cyclical displays involve a repetition of units of movement that may include various body components (Fig. 4.14a). Successive units, or cycles, may be identical, but more often they are slightly different; e.g., in *N. vitripennis* the number of head-nods per cycle changes according to a fixed pattern (Barrass, 1961). In finite displays, quality changes during the performance because movements that were included earlier are omitted, to be replaced by new components. Finite displays have a predictable end, i.e., a finale, irrespective of whether female receptivity does or does not occur at that moment. Finite displays occur in *Melittobia* species (Fig. 4.14b) and probably in four species of *Monodontomerus* (Torymidae) (Goodpasture, 1975), although no mention is made of the timing of female receptivity (Sect. 4.3.6).

The cyclical display of *N. vitripennis* males provides an excellent opportunity for a quantitative investigation of male courtship. Except for the first series, consecutive series of nods are preceded by an antennal sweep and are separated by intervals. Separate nods and series of nods can easily be counted and recorded, but between-series intervals require an automatic time-marker (Sect. 4.2.6). Additionally, males drum on the female's compound eyes with their front tarsi, and vibrate their wings (Barrass, 1960b). Drumming movements are very rapid and cannot be counted separately by eye, but can be quantified per bout, as present or absent or quantified more precisely using video tracking or slow-motion recording (i.e., using EthoVision, Sect. 4.2.6). Wing vibrations can be quantified precisely using acoustic recording (Sect. 4.2.6). The number of

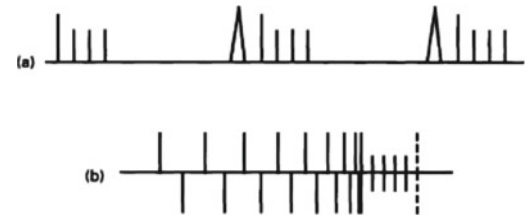

Fig. 4.14 Schematic representation of: **a** 'cyclical', and **b** 'finite' display. Time runs from left to right. The pteromalid *Nasonia vitripennis* produces a cyclical display. In **a** triangles denote antennal sweeps, vertical lines denote head-nods. Single nods are separated by short pauses and are temporally clustered. First nods are denoted by longer lines than the following nods because they are more elaborate, last longer and coincide with the pheromone release. All series except the first are preceded by an antennal sweep. Series are separated by intervals. The period from the first nod of a series to the first nod of the subsequent series is termed a courtship cycle. A cyclical display consists of a repetition of identical or nearly identical cycles. The eulophid *Melittobia acasta* produces a finite display. In **b** long vertical bars above the horizontal denote antennal movements (flagellar vibrations concluded by grasping of the female's antennae), those below the horizontal denote series of swinging movements with the hind legs in combination with a loss of antennal contact between the male and female. Alternations accelerate until they coincide. At this point (denoted by a series of shorter vertical bars above and below the horizontal) the antennae are stretched downwards and the hind legs moved up and down, rubbing against the female's thorax, instead of swinging to and fro. After a few seconds, these movements are followed by a brushing of the middle legs against the female's eyes (denoted by the dashed vertical line at the far right). A finite display does not consist of distinct, more or less identical cycles, but changes markedly over time, leading to a succession of 'phases' such as 'introduction' and 'finale'

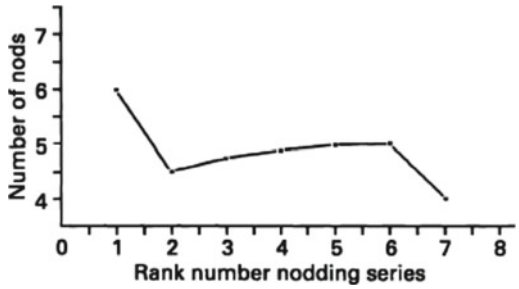

Fig. 4.15 The number of nods in successive nodding series of courting *Nasonia vitripennis* males

nods per series varies, as does the duration of intervals between series (general trends are illustrated in Figs. 4.14, 4.15 and 4.16, see also Barrass, 1960b; Jachmann & van den Assem, 1993, 1996; Dalla Benetta et al., 2021). Males exhibiting more nods per series exhibit fewer series up to the moment of dismounting. In measuring a *N. vitripennis* male's display production, the first step would be to make external conditions constant by using an unreceptive or dummy female. Despite standardisation, males differ in the duration of displays (the period between mounting and giving up) they perform, and there may also be variation within individuals observed on more than one occasion. Thus, performance is not exclusively controlled by external factors.

There are several techniques that can be used to test how such factors alter male investment in courtship. For instance, in order to test whether males 'use up' resources required for courtship, males can be introduced to females and their

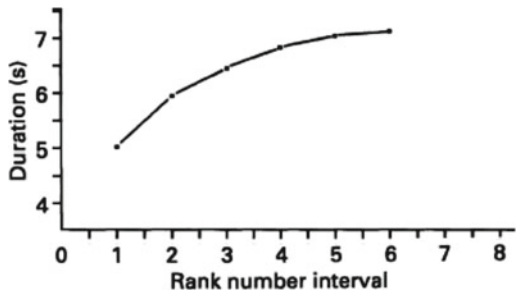

Fig. 4.16 The duration of successive intervals between head-nod series in the courtship display of male *Nasonia vitripennis*

investment in courtship recorded (e.g., time spent, number of display elements), or manipulated (i.e., courtship can be stopped early by the experimenter). The same males can then be presented with the same or a different female and their investment in the next courtship recorded in the same way. Such experiments have shown that in *N. vitripennis* the second display is always shorter than the first if the males have a break of less than 24 h, but larger differences are found with shorter pauses (Fig. 4.17). In *N. vitripennis* time is not the only influential factor: males that have produced longer first displays (i.e., with more nodding series) also produce longer second displays (Fig. 4.18). These results suggest that something is 'used up' during displays, and that 'recovery' takes place during the pause. With longer pauses there is more recovery, but the recovery rate declines with time (van den Assem et al., 1984).

Further conclusions are that males will court before they are able to display maximally, and that current display production is partly influenced by earlier displays. Thus, mating displays can be quite variable, even when performed by the same male. Clearly, for an understanding of display dynamics, records of motor patterns are insufficient; measurements of between-display intervals are also required (Fig. 4.17). However, the performance of finite displays by *Melittobia* males appears to support the notion of an automaton: males produce a sequence of displays (directed towards receptive and unreceptive females) of roughly similar duration (Fig. 4.19), independent of the duration of intervening pauses (van den Assem et al., 1982a). Other factors can influence male willingness to court, and male investment in courtship. For instance, social conditions which alter males' perceived risk of sperm competition, as well as male quality (and the quality of his potential mate) are some factors that might influence male investment in courtship (as well as post-copulatory investment; Boulton et al., 2015). The protocols outlined above can readily be modified to test how male investment in courtship varies according to these factors, for instance by presenting males with females of different sizes, or females that have previously mated (Joyce et al., 2009; King et al., 2005).

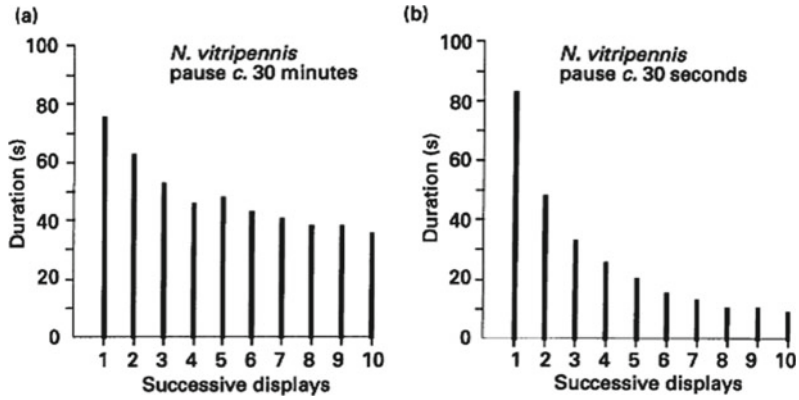

Fig. 4.17 Durations of successive displays in male *Nasonia vitripennis* inter-display intervals **a** ca. 30 min., **b** ca. 30 s. Males cannot be described as simple courtship 'automata', as they do not produce unitary displays at any time

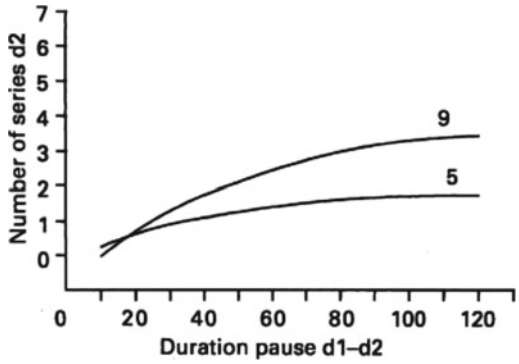

Fig. 4.18 With pauses of equal durations between a first (d1) and a second (d2) display, *Nasonia vitripennis* males that produce more (9) nodding series in the first display 'recover' more rapidly than those that produce fewer (5) series

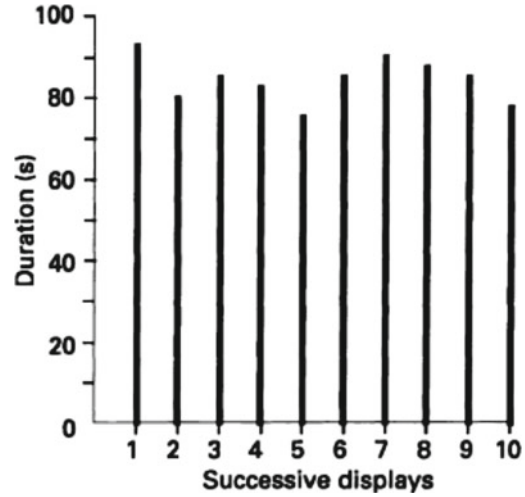

Fig. 4.19 Courtship automata: duration of successive displays in male *Melittobia acasta*

Displays typically come to an end when the male responds to external factors such as the onset of female receptivity (Sect. 4.3.6). However, males that court dummies or unreceptive females (Sect. 4.3.3) also give up for reasons other than being physically exhausted. The cessation of display coincides with (or occurs immediately after) head nodding in *Nasonia* and other species. It is possible that the readiness to court is incrementally reduced with the performance of each nod. A stiff hair struck against the courting male's dorsum (Fig. 4.20) caused cessation of display and both the timing and duration of the interruption strongly influenced the numbers of extra series and nods produced. Hence, the display behaviour of *N. vitripennis* (and presumably other species) follows simple rules (further discussed in Jachmann and van den Assem, 1993, 1996).

Van den Assem et al. (1984) found that *Nasonia* males that have courted several unreceptive females in succession produce only a short display with subsequent females, its duration depending on the length of time since previous dismounting. Males that had courted five females were either kept at room temperature (20 °C) or placed in a freezer at −30 °C (males were in styrofoam containers and thus not exposed to such low temperatures all the

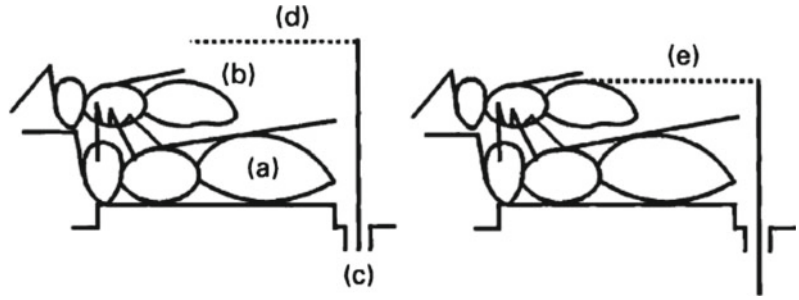

Fig. 4.20 Apparatus for interrupting male display without causing dismounting: **a** dummy female, **b** courting male, **c** opening in bottom of observation cell and vertical rod, **d** hair, **e** hair in 'strike' position

time) for 30 min and were then presented with a sixth female at room temperature. Although the 'freezer' males were initially completely immobile and appeared to be dead, they became active quite rapidly, at which time they were presented with a female. 'Room-temperature' males produced short displays as predicted, but 'freezer' males produced displays of the same length as those of inexperienced males, as if the effect of earlier performances had been lost. This rules out the possibility that something 'used up' during displays has to be either replaced or processed during pauses. If 'freezer' males were kept alone at room temperature for around five minutes before exposure to the sixth female, the effect of freezing was no longer evident. Thus, genuinely inhibitory processes, and not 'consumption' processes, appear to play a role in terminating displays. The physiological mechanisms are unknown but learning and memory may play role in this behaviour, as has been shown to be the case for host-associated behaviour in *Nasonia* (e.g., Hoedjes et al., 2012). Unorthodox procedures may thus yield results that provide new insights into the causal processes underlying mating behaviour (see Sect. 4.3.6 for an example of the mechanism underlying the switching off of female receptivity).

4.3.6 Female Receptivity

4.3.6.1 Introduction

In many insect species females must signal receptivity in order to copulate; forced copulation by males is seldom possible, particularly in parasitoids, where females must open their genital pore to permit intromission. The mere presence of a male never results in overt female receptivity. Stimuli needed for the transition from latent to overt female receptivity have been discussed (Sect. 4.3.4). Here, we consider more specific questions:

1. Is the onset of receptivity random during a display, does it relate to a particular display movement, or is it induced by accumulated stimulation reaching a threshold?
2. Are courtship sequences 'chain reactions', with both participants reacting to one another step by step until copulation?
3. Do females vary in their propensity to become receptive based on variation in the male courtship display or the male phenotype (i.e., precopulatory mate choice)?

Direct observations are required to establish whether the onset of female receptivity coincides with the performance of a particular display movement by the male. However, this may not be obvious if, in cyclical displays, males repeat identical movements at high rates. In some ichneumonids, males vibrate their wings vigorously and repeatedly attempt to grasp the female's genitalia with their claspers (this is one case where female control over mating is not complete). This 'wriggling' develops into copulation. A similar sequence is seen in *Trichogramma evanescens* (Hase, 1925; J. van den Assem, pers. comm.). Technological advances in recording and measuring behaviour and associated audio and olfactory cues (outlined in Sects. 4.2.6 and 4.3.4) can help elucidate the stimuli that induce

receptivity. Coincidences between display components and the onset of receptivity are easier to discern in cyclical displays with more differentiated motor patterns. Experiments involving mouthpart-sealed *N. vitripennis* males demonstrate a chemical stimulus emanating from the extruded mouthparts (Sect. 4.3.4). The onset of receptivity coincides with the first nod of a series (Fig. 4.21a) and (almost) never with second or subsequent nods. Thus, a periodic production of essential stimuli induces receptivity. There is additional evidence: first nods differ in movement from subsequent nods, and females are able to perceive the stimuli continuously. Females may signal receptivity at any time once a threshold concentration of pheromone is reached. With finite displays, the production of releaser stimuli seems to occur once per display, which makes the timing of the switch from latent to overt receptivity predictable (Fig. 4.21b). This is even stronger evidence of periodic production of stimuli.

Simple chain reactions can be ruled out by observations of males courting dummies (Sect. 4.3.3); males transition to the next phase of courtship without receiving cues from females. The timing of successive display cycles appears to be a matter of internal control, but external stimuli are also of importance: courting *N. vitripennis* males continuously monitor the position of the females' antennae (demonstrated with dummy females, Sect. 4.3.5).

4.3.6.2 Receptive Posture

In parasitoids, the switch from latent to overt receptivity is generally indicated by the female raising her gaster and opening the genital orifice. The posture varies between taxa. In eulophids, females 'sag in the middle' while raising the abdomen and direct the head and antennae upwards. In *N. vitripennis* and related Pteromalinae, the head is lowered and the antennal flagellae are drawn towards the front of the head (Fig. 4.22). In *Cotesia rubecula* the head is tilted forwards and the antennae are lowered (Field & Keller, 1993b) while in *Eretmocerus eremicus* females become receptive without any clear changes in antennal position, while the abdomen is lifted to expose the genital orifice (Hunter et al., 1996).

As soon as a female is receptive, the male ceases courting and switches to copulatory behaviour. The raising of the female's abdomen

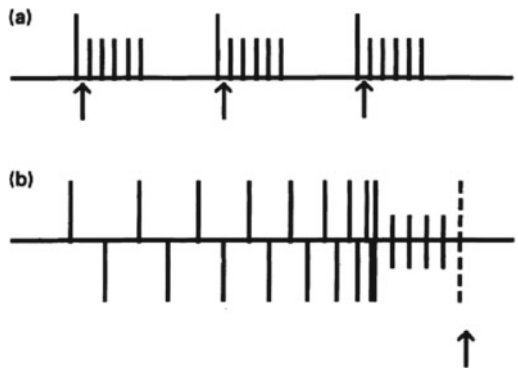

Fig. 4.21 Onset of receptivity (denoted by upward-pointing arrows, for other symbols see Figure 4.14) according to performance of a particular event: **a** in the cyclical display of *Nasonia vitripennis* receptivity may occur at several points in a sequence, but always immediately following the performance of a first nod; **b** in the finite display of *Melittobia acasta* receptivity occurs at a unique point in time, at the end of a finale

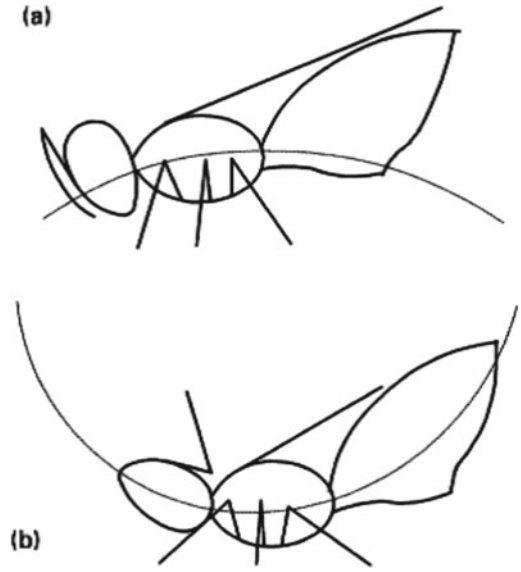

Fig. 4.22 Female receptivity posture of **a** pteromalids and **b** eulophids

may provide a tactile stimulus to a mounted courting male in many species, but this is not the case in *N. vitripennis* and related Pteromalinae (Fig. 4.23), probably because of the anterior position a male adopts on a female. However, antennal folding would be easily perceived by the male. van den Assem and Jachmann (1982) used a dummy with movable antennae, constructed from a freshly killed female, with antennae removed, and a rod bearing two pieces of wire representing antennae, which could be moved up and down (Figs. 4.24 and 4.25). Males courted the inactive dummy as they did a living female and gave up after a similar period. When the 'pseudoantennae' were moved downwards, males ceased courting and attempted to copulate. This shows that antennal movements signal receptivity to males. Similar movements in other pteromalids probably have the same function.

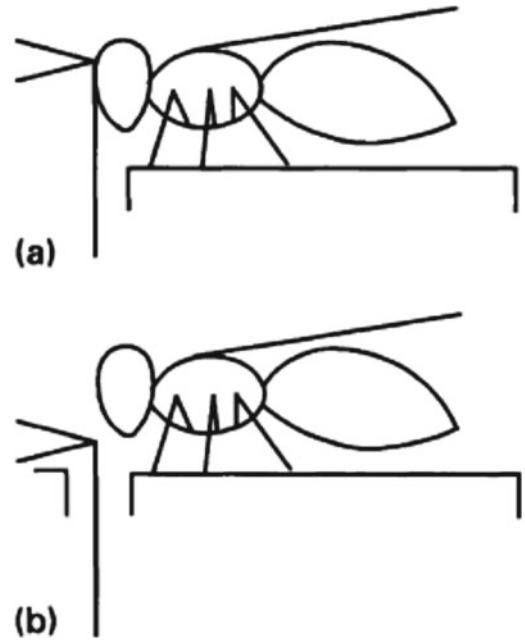

Fig. 4.24 A dummy female *Nasonia vitripennis* with movable antennae; antennae in **a** courtship-eliciting position and **b** receptive position

4.3.6.3 Intraspecific Variability in Onset

In parasitoids, virgin females will usually become receptive when courted, sometimes even after very limited male display (or even no display if olfactory cues are strong enough). Outside of the parasitoids, this is less common and even virgin females often exhibit mate choice, becoming receptive only for certain males (Kelly, 2018). Despite this there is still evidence for mate choice in parasitoids, and individual males vary in their ability to initiate female receptivity. In several species females are less likely to become receptive when courted by older males, smaller males and males that have mated more times previously (Cheng et al., 2003; He & Wang, 2008; Joyce et al., 2009; see Boulton et al., 2015, for a comprehensive review). This may relate to male pheromone depletion. Pheromones are costly for males to produce; they get used up and are not replenished as rapidly as mating opportunities arise (Ruther et al., 2009). Pheromone titres can provide females with information about whether males are likely to be sperm depleted and so male pheromone quantity can signal male quality (Sect. 4.4.4). There is greater scope for female mate choice in parasitoid species that mate more than once. Induction of further receptivity usually requires a longer display and some males are more able to overcome elevated thresholds for receptivity (Boulton et al., 2015). Polyandry (female multiple mating) also provides females with the

Fig. 4.23 Position of the courting male in different species of Pteromalidae and the possibility of the male perceiving directly the raising of the female's gaster

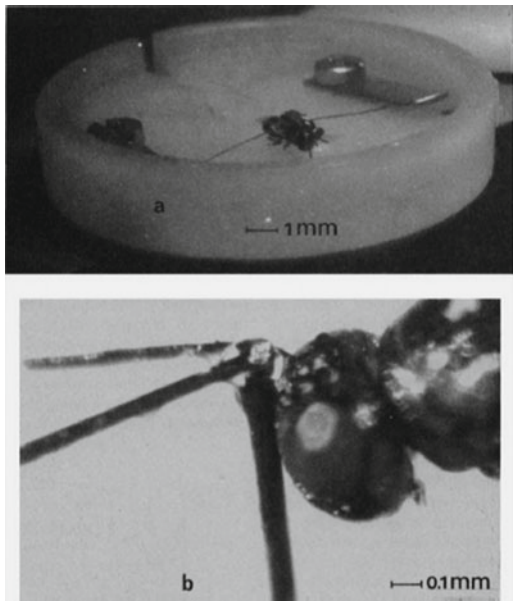

Fig. 4.25 **a** A male *Nasonia vitripennis* performing courtship behaviour on a dummy with movable antennae. **b** The dummy's head in contact with the movable antennae. Because frequent renewal was necessary, dummies were fastened into the observation cell with a human hair strung between two clips. Further details are given in van den Assem and Jachmann (1982)

opportunity to compensate for previously mating with poorer-quality mates (Andersson & Simmons, 2006).

There is also evidence to suggest that individual females differ in their sensitivity to identical stimuli: virgin female *N. vitripennis* were allowed to become receptive and copulate with a male, but the male was removed before he regained the anterior position for post-copulatory courtship (during which females usually again signal receptivity). Females were then classified as those that had signalled initial receptivity with the male's first head-nod series, and those that did so after three or more series. All females that were presented with another male after 30 min signalled receptivity, although a longer display was required to release the response. The initial inter-female differences remained: females of the first category required less stimulation than those of the second category. On the other hand, males that had induced receptivity with the first nod of the first series were not consistently more efficient compared with males that induced receptivity after several series, when presented with a second series of females half an hour later (van den Assem, 1986).

4.3.6.4 Female Mating Rate

Unlike in almost all other insects, in female parasitoid wasps sexual receptivity can usually be induced only a limited number of times: at some point further matings will be refused. Females of many parasitoid wasp species (Gordh & DeBach, 1978; Ridley, 1993; Boulton et al., 2015) but few predator species (e.g., Fincke et al., 1987; Arnqvist, 1997; Arnqvist & Nilsson, 2000) mate only once (monandry). Ode et al. (1997) conducted an experiment to examine remating by females of the gregarious parasitoid *Habrobracon hebetor* (Braconidae). Virgin females that were recessive mutants for eye colour (ivory) were presented with either an inexperienced mutant male or a wildtype male (black eyes) and were then presented with the opportunity to mate with a male of the opposite eye colour on all successive days. Males and females were placed together for 15 min during which all copulatory events were noted. After each mating period, females were kept individually and given four hosts per day until death. All progeny were reared and the eye colour of all daughters was noted to detect the occurrence of a second mating. Daughters possessed the eye colour of their fathers. Only 5% (3/64) of females re-mated despite over 60% of them running out of sperm from the first mating.

Insecticide resistance can be used as a phenotypic marker to study multiple mating (polyandry in females, polygyny in males) in a species. A polyandry assay involves mating females to insecticide-resistant and non-resistant males and then testing whether offspring are resistant, non-resistant, or a mix of both if the mother is polyandrous. This can be time-consuming both to devise and carry out, and it obviously requires that members of the population possess an insecticide-resistance gene (see Baker et al., 1998, on *Anisopteromalus calandrae*, some individuals of which show resistance to malathion). Another method is to allow

females to mate with a sterile male (chemosterilants or radiation can be used) to assay multiple mating and sperm precedence (Boulton et al., 2018a; Sect. 4.4.2). This method involves recording the proportion of fertilised eggs that successfully develop depending on which male the female mated with first (sterile or fertile). This is a straightforward assay in diploids and species that lay eggs in locations that allow them to be recovered and counted. However, in haplodiploids such as parasitoid wasps, the results can be complex to interpret, since sons are produced from unfertilised eggs (see Boulton et al., 2018a, for means of accounting for this). There are further complications in species where eggs are not clearly visible (e.g., endoparasitoids and 'gap layers' like *Nasonia* that oviposit between the pupa and the puparium), as the eggs of these species cannot easily be recovered and this method requires recording the proportion of fertilised eggs which do (fertile male) *versus* do not (sterile male) develop.

One key issue with all of the above methods is that males from phenotypically marked lines and insecticide-resistant or sterile males might represent weaker competitors in pre- and post-copulatory sexual selection. Females might be less likely to mate or re-mate with these males and their sperm might not be as successful. These experiments can be very time-consuming as, in order to draw inferences, many controls are required. These issues can be overcome by using wildtype males that are genetically variable in mating trials. Offspring can then be genotyped for quickly, and these days inexpensively, assaying multiple paternity using microsatellites or genotyping-by-sequencing approaches (Avril et al., 2019). This can be done using a pool-seq assay (where multiple individuals are pooled and then genotyped in the same reaction; Wiberg et al., 2020) or even by genotyping spermatheca contents (Demont et al., 2011). As yet, these techniques have not been widely used to assess polyandry in parasitoids (although see Awad et al., 2017 for an example in an insect predator, *Harmonia axyridis*).

4.3.6.5 Switching-Off of Receptivity

Both internal and external factors are probably involved in the switching-off process that makes females permanently or temporarily unresponsive to courtship stimuli. Those related to insemination *sensu stricto* are obvious candidates; stored sperm can provide direct, 'external' stimuli (e.g., as a measure of the fullness of the spermatheca; Thibout, 1975). However, an ejaculate contains materials other than sperm that directly or indirectly affect a female's receptivity. For instance, in some Lepidoptera, males transfer non-fertile sperm which might 'fill up' the spermatheca and reduce female receptivity (and as such, sperm competition; Wedell, 2005). In other species males transfer mating plugs which inhibit subsequent matings (regardless of female receptivity; Avila et al., 2011). More broadly, seminal fluid proteins in the ejaculate can have a variety of effects on female physiology, from reduced receptivity to future matings to increased longevity (Chen et al., 1988; Chapman et al., 1995; Avila et al., 2011; Boulton et al., 2015). Allen et al. (1994) found that post-copulatory courtship and mate guarding in *Aphytis melinus* (Aphelinidae) reduced the chance that females would mate with other males but did not completely switch off female receptivity to further mating. Field and Keller (1993a) found that female *Cotesia rubecula* remain receptive for a brief period after the first mating and that post-copulatory mimicking behaviour by the first male often distracts rival males during this period (Fig. 4.26). In *N. vitripennis,* post-copulatory courtship reduces receptivity and thus the chances that females will mate again (van den Assem & Jachmann, 1999; Ruther et al., 2010). In this species, however, after many generations in laboratory culture post-copulatory courtship becomes less effective at 'switching-off' and polyandry becomes more common (Burton-Chellew et al., 2007a).

In some insects, males transfer anti-aphrodisiac substances to females after mating. This renders females unattractive to other males and so reduces sperm competition (e.g.,

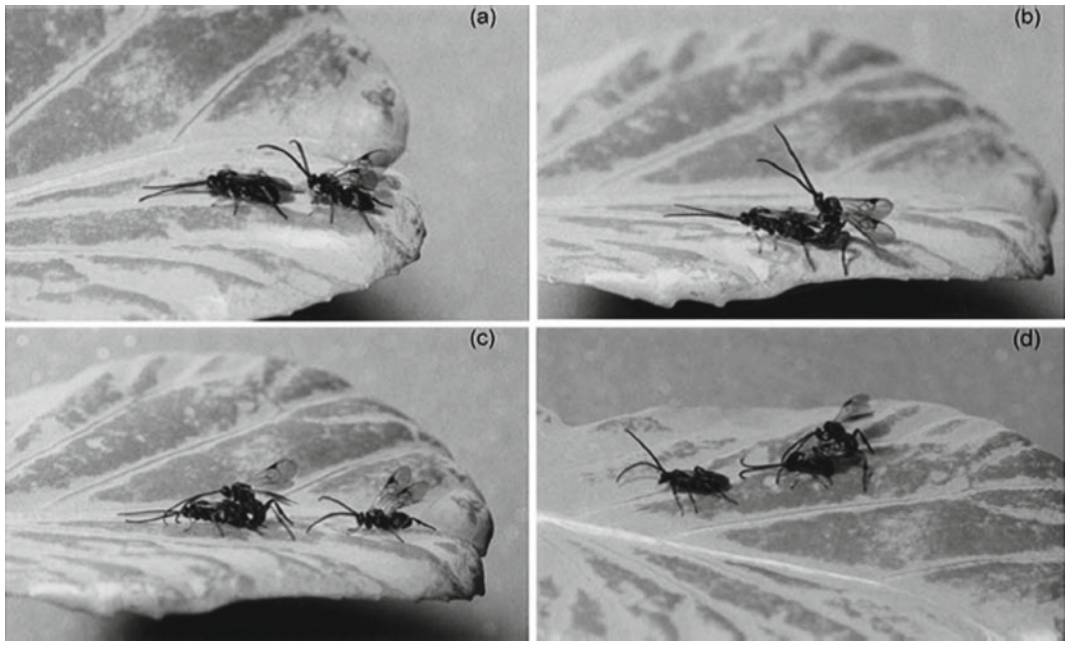

Fig. 4.26 Post-copulatory female-mimicking behaviour in *Cotesia rubecula* (Braconidae): **a** female (left) signals receptivity to courting male (right); **b** female and male in typical copulatory position; **c** copulating male (centre) mimics female receptive position by lowering antennae in response to courtship by second male (far right); **d** following copulation, female (left) moves away, whilst the second male (right) is deceived by female-mimicking behaviour of the first male (centre). Reproduced from S. A. Field and M.A. Keller (1993), 'Alternative mating tactics and female mimicry as post-copulatory mate-guarding behaviour in the parasitic wasp *Cotesia rubecula'*, *Animal Behaviour*, 46 (6), pp. 1183–1189, by permission of the publishers, Elsevier Science, W.B. Saunders Company Limited and Churchill Livingstone

Andersson et al., 2000). However, this has not been reported in the parasitoid wasps. Instead, it is the female wasps themselves that secrete anti-aphrodisiac pheromones after mating (*Spalangia endius*; King & Dickenson, 2008; Mowles et al., 2013), which correlates with the switching-off of receptivity. Other evidence for female control of receptivity comes from *Leptopilina heterotoma* (Cynipidae) and *Lariophagus distinguendus* (van den Assem, 1969, 1970), *Nasonia vitripennis* (van den Assem, 1986; Ruther et al., 2010) and *Habrobracon hebetor* (Ode et al., 1997); in these species females that had become receptive but were uninseminated (e.g., if mating was terminated before sperm transfer) were subsequently unreceptive. This suggests that becoming receptive once (even if mating is not successful) can be sufficient to inhibit re-mating. In *N. vitripennis* when receptivity is 'switched off', so is female attraction to male sex pheromones (Ruther et al., 2010). More recent work suggests that the proximate mechanism for this switch involves increased dopamine (DA) levels after mating. Lenschow et al. (2018) found that feeding mated females DA-antagonist maintained attraction to male sex pheromones, while feeding DA to virgin females rendered them non-responsive.

Broader evolutionary patterns of female receptivity in the parasitoids remain to be fully elucidated. While it seems that monandry is likely to be ancestral in the group, the existence of post-copulatory courtship and male adaptations to reduce sperm competition suggest an evolutionary history of polyandry in some species. Phylogenetic comparative studies (Sects. 1.2.3, 4.6.2, 5.3.4) will be useful in disentangling such evolutionary correlations in the future.

4.4 Copulation and Insemination

4.4.1 Male Readiness to Copulate

Males can generally copulate without first courting. If a male encounters a female in the copulation posture (a living female or a dummy obtained by killing a copulating female in liquid nitrogen), he will proceed to copulate immediately. For example, in *N. vitripennis* 'sneaky males' are able to copulate with females actively courted by another male (Fig. 4.27). Similarly, males may perform some part of the courtship sequence, but 'short-circuit' this by exploiting the courtship display of another male (Fig. 4.28).

In *N. vitripennis*, males perform post-copulatory courtship (Sect. 4.3.6), where they re-assume the anterior position immediately following copulation and proceed to court afresh, and females usually signal receptivity for a second time. However, males never respond to a second signal but remain at the front and continue displaying for a short period before dismounting. Hence males react differently to identical external signals, depending on internal state which is influenced by previous behaviour. The copulatory act causes changes: males removed prior to, or following, the first receptivity signal, or just prior to genital contact, immediately attempt copulation when again confronted with the signal. The post-copulation refractory period is short. A male that is prevented from performing a post-copulatory display (by being brushed off by the female at the termination of copulation) and which quickly mounts another female, will not back up if the second female is quick to signal (at the first nod of the first head-nod series). If a longer display is necessary to induce receptivity, the male will back up and copulate. The refractory state is thus not a female-specific, but a time-dependent, phenomenon.

Fig. 4.27 A courting *Nasonia vitripennis* couple, with a 'sneaky' male clasping the female's gaster

After mating, the reproductive success of a male depends entirely on that of his mate. One way in which males can exert direct influence on the prospects of their sperm would be by choosing larger females in preference to smaller ones, since the former tend to have a higher fecundity (Sect. 2.7.3; Bonduriansky, 2001). However, in some species larger females are more likely to re-mate (e.g., *Alabagrus texanus;* Adams & Morse, 2014) and so male choice of larger females could increase the risk of sperm competition and so reduce male reproductive success. Joyce et al. (2009) found that males prefer smaller females in *Cotesia flavipes* (but not in *C. marginiventris*), but whether this reflects sperm competition avoidance is not clear. While male mate choice based on female size has not been investigated systematically in the parasitoids (although see Antolin et al., 1995, for an example of size-assortative mating in *Habrobracon hebetor*), there is abundant evidence that the risk of sperm competition has shaped male mating preferences in the group. Male preference for virgin females has been demonstrated in *Spalangia endius*, *Trichogramma euproctidis*, *T. chilonis* and *Microplitis croceipes* (independently of differences in the behaviour of virgin and mated females; King et al., 2005; King & Dickenson, 2008; Martel et al., 2008; Makatiani et al., 2013; Wang et al., 2016). In *S. endius* this even leads to a temporary aversion to mating with any female after mating with or encountering a mated female (Fischer & King, 2012). When we consider also that post-copulatory courtship is common in the parasitoids it seems that sperm competition risk has exerted a strong selective effect on male mating behaviour and preference in the parasitoid wasps, despite low levels of re-mating by females.

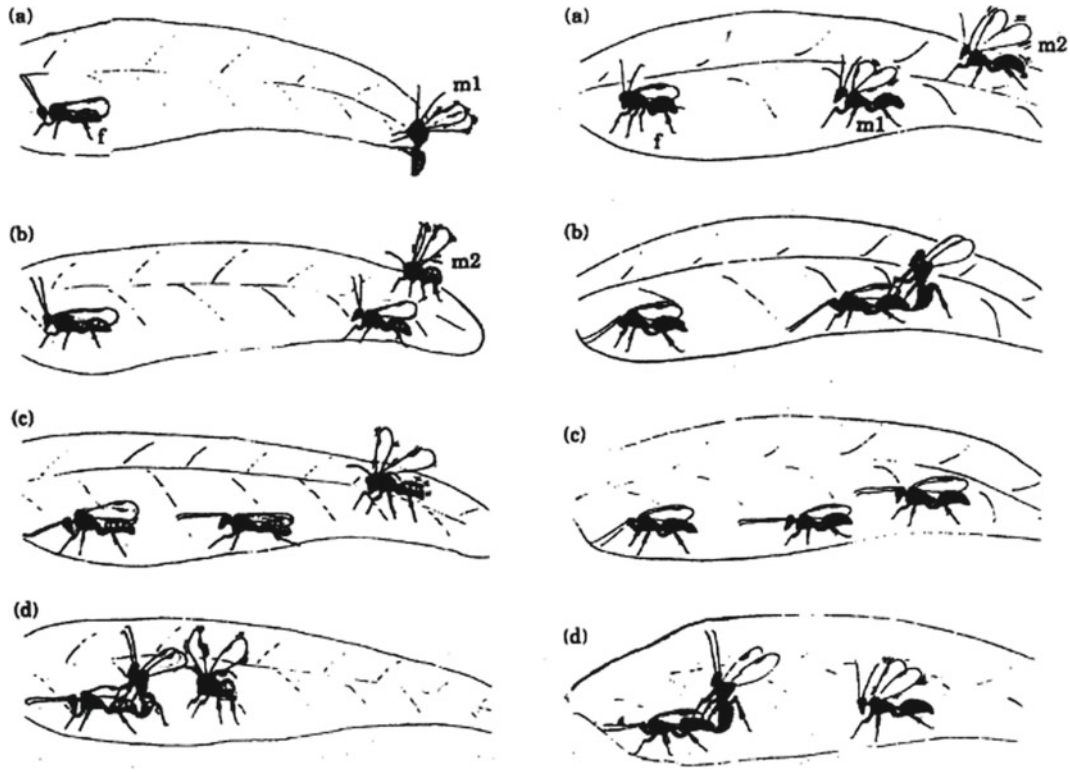

Fig. 4.28 Two mating tactics in the braconid parasitoid wasp *Cotesia rubecula*, alternative to performing full courtship (Fig. 4.2). In the left-hand sequence, a male exploits the courtship display of a rival male ('short-

4.4.2 Duration of Copulation

Males can also preferentially invest in matings with different females. Longer copulation has been assumed (and in some cases shown) to result in increased transfer of sperm and seminal fluid proteins (Kelly & Jennions, 2011). Males in some species have been shown to strategically allocate more resources to females that provide them with greater fitness returns (i.e., larger and virgin females; Kelly & Jennions, 2011). However, within pteromalid and eulophid parasitoid species, there is no current evidence that males copulate for longer with larger females. It seems that in parasitoids copulation duration is a poor proxy for ejaculate transfer. Instead it seems that copulation duration is under female control as, in several species, receptive females remain in the copulation posture for similar periods irrespective of whether a copulation has or has not occurred (van den Assem, 1969, 1970, 1986). Furthermore, while there is considerable interspecific variation in copulation duration (e.g., *N. vitripennis* 15–20s, *Lariophagus* 40–80s, *Melittobia* 5–10s [all at 22 °C, note that durations are temperature dependent]), individual males are consistent in the length of copulations for successive matings (copulation duration is not related to sperm depletion; Sect. 4.4.4). There is evidence for strategic ejaculate allocation independently of copulation duration though. For instance, in *Trichogramma euproctidis* males transfer fewer sperm when the perceived risk of sperm competition is higher (i.e., when they were reared with male rival; Martel et al., 2008). This reduces male investment in matings when the outcome (in terms of paternity) is uncertain (i.e., males may sire few, or none, of a female's offspring under sperm competition).

4.4.3 Ejaculation

Prior to ejaculation, mature sperm pass from the testes into two pairs of chambered vesicles. The proximate pair are smaller and thicker walled than the distal pair and open into the ejaculatory duct. There is a sphincter muscle between the chambers. The proximate chambers become empty immediately after mating (Wilkes, 1965), and their combined volumes can be taken to correspond to a 'full ejaculate'. The minimum intervals between inseminations suggest that the proximate chambers can be refilled rapidly. A male is expected to invest his entire sperm production in one ejaculate only when mating occurs once per lifetime. Honeybee drones, for example, are lethally damaged by ejaculation and die shortly afterwards. A similar phenomenon would be expected in predator species where males are certain to be consumed when copulating (Sect. 4.4.2). More generally, males are predicted to invest their ejaculates strategically depending on their expected fitness payoff for a given mating, which can depend on the number of times a male expects to mate (i.e., the above examples), the fecundity of the female, or the risk and intensity of sperm competition (Sect. 4.4.2; Parker & Pizzari, 2010). As with all insects, male ejaculate in parasitoids comprises sperm and secretions from accessory glands (Leopold, 1976; Avila et al., 2011).

In *N. vitripennis* the length of the male's protruded penis (aedeagus), in relation to the distance from the external opening of the vagina to the opening of the spermathecal duct, suggests that sperm are deposited at the duct's opening (Sanger & King, 1971). Sperm are found in the female's spermatheca within one minute of the termination of genital contact. In a number of parasitoid species, a mass of sperm swimming within the spermatheca can be observed under a light microscope at 100–1000× magnification (Wilkes, 1966; Nadel & Luck, 1985; Hardy & Godfray, 1990; Ode et al., 1995; Kazmer et al., 1996; Heimpel et al., 1997; Fig. 4.29). Sperm may move from the spermatheca's duct opening to the interior either by peristalsis or in response to a chemical gradient. There is movement in the opposite direction once sperm are used during fertilisation (King, 1962; King & Ratcliffe, 1969). Ejaculate size, and the number of ejaculates in the female spermatheca can, however, disrupt this process. In female parasitoids there is only one way in and one way out of the spermatheca and there is evidence that polyandry can delay sperm processing times in *N. vitripennis* (Boulton & Shuker, 2015; Boulton et al., 2018a, 2019) and *Anaphes nitens* (Santolamazza-Carbone & Pestaña, 2010). Constraints on processing multiple ejaculates may be another reason why the parasitoids, unlike almost all other insects, show such low rates of female multiple mating (Boulton et al., 2018a).

4.4.4 Sperm Depletion in Males

In general, males are able to inseminate more than one female. In species which produce female-biased sex ratios due to local mate competition (LMC; Sects. 1.11.2 and 5.4.2), males generally have the ability to inseminate more females than only their sisters under high LMC (see Martel et al., 2016 for a discussion of parasitoid insemination capacity under LMC). However, male parasitoids that copulate with a number of females in rapid succession may temporarily or permanently deplete their supply

Fig. 4.29 Band width of the sperm layer inside the spermatheca of *Pachycrepoideus vindemmiae* (Pteromalidae). Shown are spermatheca dissected from females that were inseminated by males that had mated previously with 2, 4, 6, 8 or 10 females (from left to right) in quick sucession. From Nadel and Luck (1985)

of sperm (Wilkes, 1963; Laing & Caltagirone, 1969; Gordh & DeBach, 1976; Nadel & Luck, 1985; Ode et al., 1997; King, 2000; Damiens & Boivin, 2006; Bressac et al., 2008; Steiner et al., 2008; Boivin, 2013; Abe, 2019; Fig. 4.29). In some parasitoid wasp species, males are 'synspermatogenic', i.e., the sperm supply is replenished after a period of no mating, but these males tend not to fully regain their initial insemination potential, and full depletion reappears rapidly (Laing & Caltagirone, 1969; Ode et al., 1996). In at least two species, *N. vitripennis* (van den Assem, 1996) and *Habrobracon hebetor* (Ode et al., 1996), larger-sized males have a greater insemination capacity. Males that copulate infrequently are depleted of sperm less rapidly than males that copulate frequently. Male *Pachycrepoideus vindemmiae* that copulate at 30-minute intervals reach a state of equilibrium where production equals ejaculation (Nadel & Luck, 1985). In other species males are 'prospermatogenic'; they emerge with their full complement of sperm and once sperm depleted cannot replenish their sperm supplies (Boivin, 2013).

The extent to which sperm depletion occurs in the field is largely unknown. The one exception we are aware of comes from a field study of *Habrobracon hebetor*: approximately 20% of the females were found to have no sperm in their spermathecae (Ode et al., 1997). Many of the females brought into the laboratory, where they were given hosts daily until death, quickly ran out of sperm (as evidenced by the production of only sons). That most females refused to re-mate, even after their supply of sperm was exhausted, suggests that sperm depletion is a biologically relevant phenomenon in this species.

Parasitoid wasps are emerging as a model system to study the evolution of sperm production and the effects of sperm depletion on females due to the variation in spermatogeny in the group (Boivin, 2013; Martel et al., 2016). Sperm depletion and replenishment can be assayed in a number of ways in parasitoids. The most direct approach involves counting sperm in the male seminal vesicles after successive matings or 'rest' periods (Bressac et al., 2008). Alternatively daughter production and sperm storage by females can be used to assay sperm depletion as well as considering its effects on females (Nadel & Luck, 1985; Ode et al., 1996; Fig. 4.29 illustrates how to assess sperm storage in the female spermatheca).

In several parasitoid species sperm-depleted males have been found to continue to mate (King, 2000; Damiens & Boivin, 2006; Steiner et al., 2008; Abe, 2019). If females re-mate they can replenish their sperm supply (Damiens & Boivin, 2006) but the consequences of this for females can be severe when they are monandrous as they will be constrained to produce only sons (unfertilised eggs; Abe, 2019). In *Trichogramma evanescens* females do re-mate after mating with a sperm-depleted male. Damiens and Boivin (2006) suggest that in this species sperm-depleted males benefit from continuing to mate because doing so decreases a female's ability to store the sperm of their rivals, providing them with a relative fitness benefit. There is some evidence that male sperm depletion might favour the evolution of polyandry in the parasitoids; Ridley (1993; see also Hardy, 1994) showed that gregarious parasitoids tend to be polyandrous more often than solitary species. One reason for this may be that gregarious species have higher sperm requirements as these species lay more eggs than solitary species and often produce female-biased sex ratios due to LMC (and so require more sperm to lay more fertilised eggs, and hence daughters). Another way that females can counter the risk of mating with sperm-depleted males is through mate choice. In *N. vitripennis* females are less attracted to the anal gland secretions of sperm-depleted males as in addition to their reduced sperm reserves, these males also secrete less of this volatile pheromone (Ruther et al., 2009). In other cases, females are more attracted to/preferentially mate with large males (which can have greater sperm reserves; Joyce et al., 2009; Blaul & Ruther, 2012, see also Henter, 2004) and younger males (which are less likely to be sperm depleted; He & Wang, 2008). However, in many cases female parasitoids show no response to the risk of sperm depletion, either in terms of re-mating or exhibiting mate choice

(e.g., King, 2000; Steiner et al., 2008; King & Bressac, 2010), which may suggest that sperm depletion is not sufficiently common to exert selection on female strategies to overcome it.

4.5 Post-mating Events

4.5.1 Sperm Use by Females

In several species, females cannot use sperm immediately following insemination (Mackauer, 1976; van den Assem, 1977); the period during which females are unable to fertilise eggs can vary considerably between individual female *N. vitripennis* (van den Assem & Feuth de Bruijn, 1977) and can depend on how many times they have mated (Boulton et al., 2018a). Newly emerged females may have no mature eggs in their ovaries (i.e., are extremely synovigenic, Sect. 2.3.4), so temporary post-insemination constraints may have little effect on their subsequent reproduction. In *N. vitripennis*, the anatomy of the spermathecal duct is such that only one sperm is likely to descend at a time (Wilkes, 1965; see also Sect. 4.4.3 and Boulton et al., 2018a, for the consequences of this on daughter production after single and multiple matings). The possibility that females can exhibit cryptic female choice, biasing their sperm use towards preferred males, has been widely discussed (Firman et al., 2017) but as yet there are no examples from parasitoid wasps. Where females do mate multiply, it is likely that the relatively simple method of sperm storage (a single, spherical spermatheca and a single duct) constrains post-copulatory female choice. However, females could exert some control over sperm use if they have the opportunity to mate sequentially and oviposit over a time frame that sperm mixing can occur. For instance, when female *N. vitripennis* mate twice in quick succession, only the first male to mate sires any offspring (van den Assem & Feuth de Bruijn, 1977). If there is a sufficient delay between matings the sperm of both males are used and sperm mixing occurs to a greater extent after an initial bout of egg laying (Boulton et al., 2018a).

4.5.2 Sperm Competition, Displacement, and Precedence

4.5.2.1 Introduction

Sperm competition is the selective force that arises whenever the sperm of two or more males overlap in time (and usually in space) in a single fertile female (Parker, 1970a). Insects are predisposed to particularly high levels of sperm competition because females generally store sperm in the spermathecae, and they are usually polyandrous. Once the preconditions for sperm competition are met, the stage is set for the evolution of two antagonistic suites of male fitness traits (Parker, 1970a): offensive and defensive traits. Defensive traits work by reducing the risk of sperm competition. For instance, mate guarding and mating plugs prevent females from receiving a second ejaculate and so can prevent sperm competition arising in future (Chaudhary et al., 2017; Klug, 2018; Kvarnemo, 2018; Stockley et al., 2020). Males can also transfer anti-aphrodisiac compounds that render females unattractive to their rivals (Laturney & Billeter, 2016), or seminal fluid proteins that reduce the female's receptivity to subsequent matings (Reimann et al., 1967; Thornhill, 1976; Avila et al., 2011). In parasitoids, post-copulatory courtship can serve to reduce female receptivity to re-mating (Sect. 4.3.6), suggesting that it functions as a defensive sperm competition trait in some species.

Offensive traits serve to increase the fertilisation success under sperm competition. Perhaps the most spectacular offensive traits are the spinose appendages on the penis of certain damselflies (Fig. 4.30) and dragonflies that are used to physically remove the sperm of rivals (stored in the female) before insemination (Waage, 1979a, 1979b; Miller, 1984; Siva-Jothy, 1984). By removing rival sperm these males ensure they fertilise most, if not all, the eggs their mate lays in the subsequent oviposition bout. There are several lines of evidence that suggest variation in penis morphology in damselflies is related to variation in reproductive function which, in turn, is related to variation in the details of the mating

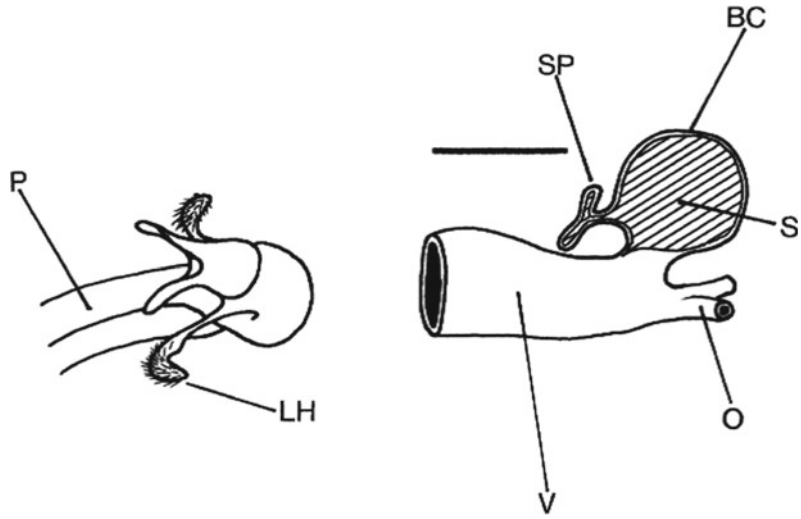

Fig. 4.30 Genitalia of the damselfly *Mnais pruinosa pruinosa.* Left panel**:** distal part of the male intromittent organ. P = penis shaft; LH = lateral horns. The lateral horns bear recurved spines which the male uses to remove the sperm of rivals stored in the female's bursa copulatrix. Left is anterior and up is ventral. Right panel: A diagrammatic representation of the female internal genitalia. V = vagina; O = oviducts, SP = spermathecae; BC = bursa copulatrix; S = stored sperm (hatched). Left is posterior and up is dorsal. Scale bar = 1 mm. (after Siva-Jothy & Tsubaki, 1989, by permission of Springer Verlag)

system (Siva-Jothy, 1984; Waage, 1984; Robinson & Novak, 1997). Offensive and defensive traits evolve under post-copulatory sexual selection, which has been well documented and comprehensively reviewed (Thornhill & Alcock, 1982; Smith, 1984; Simmons & Siva-Jothy, 1998; Birkhead, 2010; Parker, 2020).

Traits that evolve under sperm competition result in the non-random use of sperm received by a female from multiple males. Without mechanisms to bias their own sperm use, there are three possible outcomes of sperm competition. First, sperm can be randomly mixed in the female reproductive tract and the outcome of is the equivalent of a 'fair raffle'; males' sperm are used in proportion to how many are transferred (Birkhead & Hunter, 1990; Parker, 1990). This can lead to selection on ejaculate size; by transferring larger ejaculates males can increase the proportion of offspring they sire (Birkhead & Hunter, 1990; Parker, 1990). The second and third outcomes are first (P_1) and second (P_2) male sperm precedence. These mean that the first male to mate or the last male to mate sire the majority of a female's offspring, respectively. First male precedence (P_1) is the norm in the Hymenoptera (although for an exception see Elagoze et al., 1995). This is likely due to the spherical shape of the spermatheca; in species with a more tubular spermatheca the last male (P_2) to mate typically sires the majority of a female's offspring because sperm become stratified when stored, and the first ejaculates received are typically the last to be used (Ridley, 1989). Patterns of sperm precedence can be estimated using the sterile male technique or genetic markers (see below); these require the use of reciprocal mating treatments to account for the difference in competitive ability between sperm marked in different ways (Boorman & Parker, 1976).

One interesting feature of the P_2 values measured for insects to date is that they show considerable intraspecific and interspecific variation (Lewis & Austad, 1994; Simmons & Siva-Jothy, 1998). Careful comparisons (controlling for phylogeny, Sects. 1.2.3 and 5.3.4) of this variation across insect species have revealed that it is negatively associated with the species-specific mean of P_2: in other words, species with strong last male sperm precedence (high P_2, close to

1.0) have low variance (Simmons & Siva-Jothy, 1998). In general, species with high P_2 values (such as damselflies, e.g., Hooper & Siva-Jothy, 1996) have sperm competition mechanisms that completely negate rival sperm (e.g., Waage, 1979a). The scope for variance in P_2 values is therefore greatly reduced. In species with intermediate P_2 values the sperm from both males are present in the female, so the scope for variation is greater, either through passive effects such as sperm mixing, or through other undocumented effects such as cryptic female choice or interejaculate competition. Finally, the males of species with low P_2 values (first male precedence, P_1) tend to use mating plugs or other defensive tactics to protect their genetic investment. If the plug remains intact or females are rendered completely unreceptive to re-mating, subsequent males cannot mate, so P_2 remains low. However, if the plug is breached (by traits also selected for under sperm competition) or females re-mate, P_2 increases dramatically, resulting in the largest variance (Simmons & Siva-Jothy, 1998). There is some evidence that females can bias sperm precedence to exercise choice over the sperm of favoured males (Eberhard, 1991; Birkhead, 1998), for instance by ejecting the sperm of non-preferred males (Lüpold et al., 2013).

Another way of understanding why P_2 values vary is to clarify the nature of the mechanism underpinning sperm precedence. This problem has been approached through experimental and/or comparative studies of reproductive anatomy and physiology (Siva-Jothy, 1984; Siva-Jothy & Tsubaki, 1989; Miller, 1991; Robinson & Novak, 1997; see above regarding spermathecal shape). Such studies usually combine anatomical studies of male and female genitalia and their disposition during copulation with manipulations, or observations, aimed at identifying sexually selected function during copulation. Because of the immense diversity of insect genitalia (almost certainly reflecting a similarly immense range of copulatory and sperm competition mechanisms) there is no single methodological approach for examining the mechanistic basis of sperm competition. However, examining the structure, dimensions, and articulations of the male genitalia usually provides valuable insights into the operational constraints and abilities during copulation and so may exclude, or suggest, certain mechanistic options. Moreover, male genitalia are often well documented and described because of their importance in taxonomic studies. The dissection of *in copula* pairs that have been snap-frozen in liquid nitrogen at different times during copulation then provides some idea of how the genitalia of males and females interact, further narrowing down mechanistic possibilities, and usually giving considerable insight into the function of otherwise abstract structures (Crudgington & Siva-Jothy, 2001; Dougherty & Shuker, 2015). Because the female genitalia concerned with sperm competition are internal and consist mainly of endocuticle, their structure is better documented with careful dissection as well as serial sectioning of wax-embedded samples. Samples for sectioning should have sclerotised cuticle removed, be fixed in Bouin's fixative, and be subsequently embedded and sectioned (Barbosa, 1974; Bancroft & Stevens, 1991). Staining the sections with haematoxylin and eosin provides good histological resolution in most insect tissues and, when combined with the spatial information inherent in the serial sections, will provide information to reconstruct the form, and to some extent histological function, of the female genital tract ('visualisation' software is now available, allowing serial section data to be converted into an integral whole). In species with visual mutants (e.g., eye colour), accurate estimates of P_2 can be generated such as seen in a study of *Habrobracon hebetor* (Ode et al., 1995, 1997). Fluorescent labelling of sperm is one of the most useful and powerful techniques to view how the sperm of different males are being used at various stages after copulation takes place. This technique involves creating transgenic lines where male sperm express green (and red) fluorescent protein (GFP/RFP; Manier et al., 2010; Marie-Orleach et al., 2014). By dissecting out female reproductive tracts after female *Drosophila melanogaster* had mated with RFP and

GFP males, Manier et al., (2010) showed that patterns of sperm use reflect both female choice of sperm (see below) and male–male sperm competition.

4.5.2.2 Sperm Competition in the Odonata

The Odonata provide important model organisms for empirical studies of sperm competition mechanisms largely because of several unique features of their mating system and reproductive anatomy that lend them to studies of this sort (Córdoba-Aguilar et al., 2003). Moreover, the mechanism they use to achieve sperm precedence is relatively easy to quantify. Using specialised penis morphology prior to insemination, male odonates displace the sperm of rivals within (Siva-Jothy, 1987; Miller, 1991; Córdoba-Aguilar et al., 2003) or outside (Waage, 1979a), the female's sperm storage organ. For example, examination of the intromittent organ of male calopterygid damselflies (Fig. 4.30) revealed a spine-covered tip that is of the correct length and dimensions for entering the female's sperm storage organs during copulation. The male's spines are often covered with sperm after copulation, and Waage (1979a) hypothesised that they are used to physically remove the stored sperm of rival males from the female's sperm storage organ prior to insemination. Waage (1979a) predicted that if copulating pairs were interrupted at different times during copulation, a diminishing sperm store in the female would be observed, followed by an abrupt increase (ejaculation). This is exactly what was found: at one point, females *in copula* had no sperm in their spermathecae. The only logical conclusion that could be drawn was that males were removing rival sperm from storage within the female before they inseminated her with their own sperm. Subsequent sperm precedence and molecular genetical analysis (Hooper & Siva-Jothy, 1996; Siva-Jothy & Hooper, 1996) confirmed this inference. By displacing rival sperm from the place where it will be used to fertilise eggs during oviposition, and then placing their own sperm in that position, males ensure a high level of P_2 in the eggs the female subsequently lays. However, complete sperm removal does not appear to be the rule as several odonates exhibit partial sperm removal (McVey & Smittle, 1984; Siva-Jothy, 1987; Siva-Jothy & Tsubaki, 1989; Michiels, 1992; Siva-Jothy & Hooper, 1996; Córdoba-Aguilar et al., 2003). Last male sperm precedence is still high in the eggs the female lays immediately after copulation, but declines with subsequent egg batches (eggs are laid in discrete clutches at approximately 3–5-day intervals) if she does not re-mate (Siva-Jothy & Tsubaki, 1989) (Fig. 4.31). Females commonly re-mate prior to oviposition in most of the species of dragonfly studied as the mating system involves male guarding of oviposition resources (Sects. 5.4.5 and 5.5.1). Female oviposition without re-mating does occur in species where males cannot defend all the available oviposition

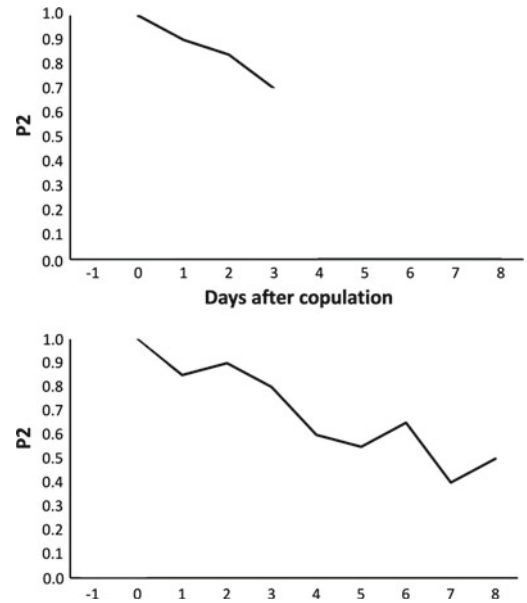

Fig. 4.31 The decline in last male sperm precedence (P_2) with time since the last copulation. Upper panel: The libellulid dragonfly *Nanophya pygmaea*. Males add their sperm to that of other males already in storage in the female's sperm storage organ (Siva-Jothy, pers. obs., after Siva-Jothy & Tsubaki, 1994). Lower panel: The calopterygid damselfly *Mnais pruinosa pruinosa*. Males partially remove the sperm of rivals that are stored in the female's sperm storage organs (Siva-Jothy, pers. obs., after Siva-Jothy & Tsubaki, 1989, by permission of Springer Verlag)

resources, and it appears that some females actively seek out undefended resources, or they avoid re-mating. In some cases, females choose where to oviposit (Hooper & Siva-Jothy, 1997), avoid re-mating prior to oviposition and are able to alter patterns of sperm precedence, using sperm from matings other than their last one when they avoid re-mating (Siva-Jothy & Hooper, 1996), for instance by ejecting sperm independently of male effects (see Córdoba-Aguilar et al., 2003, for a review).

4.5.2.3 Post-copulatory Sexual Selection and Cryptic Female Choice

Historically, the majority of studies have focused on male-based traits under selection from sperm competition, although in the last 30 years the 'polyandry revolution' has seen a shift towards understanding female perspectives in sperm competition and sexual conflict (Gowaty, 1994; Pizzari & Wedell, 2013). Females can benefit from biasing reproductive decisions in their favour. Female perspectives are not only evolutionarily important, they are also ecologically and economically (in the case of natural enemies) important (Hardy & Ode, 2007; Ode & Hardy, 2008). By exerting control over the genetic diversity of offspring, female mating behaviour and pre- and post-copulatory mate choice can protect populations against extinction and render natural enemy populations more sustainable (Holman & Kokko, 2013). In recent years a more balanced view of mating systems and mating behaviour is beginning to emerge, that considers the mating system from the interacting perspectives of males and females (Boulton et al., 2018b). This expansion in perspective has led to the realisation that females should, and can, bias paternity towards particular males, exhibiting mate choice after copulation (post-copulatory sexual selection). Eberhard (1985, 1991, 1996, 1997) was one of the first proponents of such 'cryptic female choice' (CFC), arguing that much of the within-species variation in P_2 likely reflects such post-copulatory choice by females of the sperm of particular males. Eberhard (1985, 1991, 1996, 1997) proposed that complex male genitalia, and complex and prolonged copulations, evolved as courtship traits upon which females base decisions about subsequent sperm usage.

Given the dynamic nature of sexual selection and the 'cryptic' nature of CFC it can be challenging to demonstrate that females are selecting among sperm to use for fertilisation. Birkhead (1998) outlined criteria that need to be fulfilled to demonstrate that cryptic female choice, rather than sperm competition or selective abortion/mating failure, results in observed patterns of paternity. To rule out effects of selective abortion or mating failure any undeveloped eggs or failed embryos must be counted. Ruling out the former (sperm competition) is more complex and requires reciprocal experimental designs. To do this in insects multiple females are inseminated by a pair of males (the order of mating is altered) and variation in P_2 is measured. If certain males gain greater paternity regardless of mating order, then there is evidence that they are superior sperm competitors. This design can be extended to partition variance in P_2 into male and female effects. To do this, multiple females are mated with the same males in the same order, then the effects of mating order, male identity and female identity on paternity can be established. Large effects of female identity on the variance in P_2 can suggest cryptic female choice (Parker, 1998, and references therein) and repeatability in the paternity success of individual males suggests variation in male sperm competitiveness.

Since the key work of Eberhard (1996) the study of post-copulatory sexual selection and cryptic female choice has flourished (see Firman et al., 2017 for a comprehensive review). Cryptic female choice (CFC) remains difficult to clearly and unambiguously demonstrate but much progress has been made in understanding the mechanisms that females can employ to bias sperm use. While CFC has been suggested in some predators of insects (i.e., the orb-web spider *Argiope lobata*; Welke & Schneider, 2009), for insect parasitoids assaying P_2 is made more complex due to haplodiploidy and in some cases endoparasitism (Boulton et al., 2018a; Sect. 4.3.6) as well as

sperm depletion which can cause issues in prospermatogenic species if males need to be re-used.

4.5.2.4 Measuring Sperm Precedence

As discussed in Sect. 4.3.6, sperm precedence values can be measured alongside female re-mating rates using females that have been doubly mated to two males whose offspring can be easily identified, either because the males (or their sperm) have obvious phenotypic markers or because one male is sterile. As mentioned above, one issue with these techniques is that sperm from different classes of males may be inherently better or worse at fertilising a female's eggs. To control for this, reciprocal crosses between pairs of males (each bearing one marker) are needed. By calculating the mean value of sperm precedence from two females who mate with each type of male in a different order (i.e., wildtype or sterile male first, mutant or fertile male second and *vice versa*) it is possible to control, to some extent, the effect of differential fertilisation ability.

The sterile male technique

The sterile male technique involves exposing males to a dose of gamma- or X-irradiation sufficient to induce complete, or near-complete sterility, but insufficient to affect the males' behaviour. The minimum effective sterilising dose (MESD) can be determined by subjecting groups of males to increasing doses of radiation, mating them to virgin females and then scoring the percentage hatch among the resulting eggs. The relationship between percentage hatch and dose takes the form of a decelerating curve: the MESD is the inflection point where maximum sterilisation meets the minimum dose to achieve sterilisation. Once the MESD has been determined, virgin males can be assigned to either the sterile (S) or normal (N) groups. Sterile males are exposed to the MESD. Four types of treatment are then conducted: virgin females are doubly mated to pairs of virgin males who are drawn from groups to result in the following pairwise treatments: SS, NN, SN or NS. Each male in a treatment copulates once in the specified mating order. The re-mating interval should reflect natural re-mating intervals, or the specific re-mating interval of interest: re-mating intervals can have a strong influence on the pattern of sperm precedence achieved (Simmons & Siva-Jothy, 1998; Boulton et al., 2018a), so laboratory studies need to control, and justify, this variable. Eggs are collected and scored as either sterile or normal at a point in embryological development when this distinction is clear (usually when eye-spots are developing in the embryo). In some insects including many parasitoids (particularly endoparasitoids and gap layers) eggs cannot always be reliably recovered from the oviposition site and so this must be estimated based on adult emergence: additional single-mated controls are needed to estimate the ability of sterile and normal males to produce adult offspring (Boorman & Parker, 1976; see Boulton et al., 2018a for an example of how to do this in *N. vitripennis*). Males exposed to the MESD produce sperm that bear dominant lethal mutations, so the zygotes they produce never develop past the earliest embryonic stages and rarely develop eye-spots (and do not emerge as adults). Once the eggs have been scored as sterile or normal, P_2 can be calculated from Boorman and Parker's (1976) equation: $P_R = (1 - x/p) + z/p \times [1 - (x/p)]/[1 - (z/p)]$, where P_R = proportion of eggs fertilised by the sterile male, p = fertility after NN mating, z = fertility after SS mating, and x = the number of viable eggs after an NS or SN mating. A remaining methodological problem is that the fertility of supposedly sterile males may increase over time following irradiation; this should thus be controlled for since it can lead to error in estimating P_2 (overestimation if the irradiated male was the first to mate, and underestimation if the irradiated male mated second) (Rugman-Jones & Eady, 2001).

Genetic and molecular genetic markers

The use of genetic markers, such as eye colour, for sperm competition studies requires that the phenotypic markers are discontinuous Mendelian traits. Once inheritance characteristics have been determined, the expressed phenotypes can be used as markers. As long as the pattern of

inheritance allows unambiguous assignation of offspring from controlled parental matings, then the marker can be used. An ideal situation occurs when the markers are the dominant and recessive allele at a single Mendelian locus. In such a system, virgin females expressing the homozygous recessive phenotype are mated with homozygous recessive and homozygous dominant males. Offspring can be easily assigned to the appropriate father, and the relative effect of mating order assessed.

Transgenic lines of males which have sperm that are phenotypically different can also be used to gain more detailed information about how the mechanisms underlying patterns of sperm precedence arise (Manier et al., 2010, and see above 4.5.2). It is important to test whether males from certain lines vary in their sperm competitive ability as inbreeding of marked lines and genetic correlations between the marker trait and other traits involved in sexual selection can alter natural outcomes of sperm competition (Holmes, 1974). It is now common practice to use molecular genetic techniques to assay multiple paternity and patterns of sperm precedence. Microsatellite markers and genotyping by sequencing are particularly useful for assaying paternity (see Sect. 5.3.3 for more detail on these techniques) and allow paternity of a female's offspring to be determined from laboratory studies (where mating order and timing is controlled) as well as in natural populations.

4.5.2.5 Summary

There is a vast theoretical and empirical literature concerned with post-copulatory sexual selection in insects, and how it has resulted in selection on male and female traits to improve fitness (sometimes at the expense of their mate in the case of sexual conflict) through allocating ejaculates efficiently or selecting sperm from the best males. Post-copulatory sexual selection arises because females mate multiply and because of how female reproductive anatomy can alter sperm precedence patterns. Female mating behaviour and physiology has a profound effect on male mating behaviour prior to, during and after copulation. The adaptive value of many reproductive behaviours is often only clear once viewed in the context of sperm competition and the ecology that determines the mating system.

4.5.3 Mating Frequency and Longevity

In males, frequent mating has often been found to reduce longevity, indicating that there is a cost, in terms of survival, to reproduction (see Sect. 2.8 for practical advice on measurement of longevity). In parasitoids and insect predators there is evidence that mating is costly to males as male longevity declines with mating frequency (Gülel, 1988; Burton-Chellew et al., 2007b; Perry & Tse, 2013; Charrat et al., 2023), but not in all species studied (e.g., *Habrobracon hebetor*; Ode et al., 1996). For females the opposite can also occur; females that mate more times live longer. This might reflect condition-dependent mating rates (larger females in better condition live longer and have greater sperm requirements and so mate more times). However, a common pattern across the insects is that males transfer nutritious spermatophores or other nuptial gifts to females during mating. By mating with multiple males, females can gain valuable resources that can increase their fecundity and longevity. Female mating rates tend to be higher in insect and invertebrate species where males provide such nuptial gifts, suggesting that the female mating rate evolves in response to these 'direct' benefits accrued through multiple matings (Arnqvist & Nilsson, 2000). Male ejaculates can also cause harm to females, and some seminal fluid components have been shown to reduce female longevity (Sirot et al., 2015). Males can benefit from harming females as this can increase their success under sperm competition (Sect. 4.5.2). This appears to occur in some species, where male harm (be it chemical or mechanical) reduces the likelihood that females will re-mate (Sirot et al., 2015).

In parasitoid wasps there is limited evidence that mating reduces female longevity or that mating harms females, but multiple mating can increase longevity in some species (Jacob &

Boivin, 2005; Boulton & Shuker, 2015). Likewise, many insect predators have mating systems involving nuptial gift giving (e.g., many spiders) or spermatophore transfer (ladybird beetles) by males, with the result that female longevity can increase with the mating rate, while male longevity declines (e.g., Perry & Tse, 2013). In the most extreme cases it has been proposed that the male himself is the nuptial gift in species where sexual cannibalism occurs. Examples include some spiders and mantids, where females will prey on the male during copulation. Despite the obvious survival costs to males, allowing themselves to be cannibalised could provide them with greater paternity assurance as in some cases the male aedeagus remains inside the female and acts as a mating plug, reducing the risk of sperm competition (Birkhead et al., 1988; Wilder et al., 2009; although see Lelito and Brown, 2006 for more nuanced discussion).

4.5.4 Mating and Egg Production

For diploid insects (in which all eggs need to be fertilised to produce progeny) insemination is likely to have a marked effect upon the behaviour of the female, releasing host-finding behaviour. However, insemination in haplodiploid insects such as parasitoid wasps (which do not need to mate to produce offspring) is not necessarily expected to alter female behaviour significantly. No differences in oviposition behaviour are apparent between virgin and mated females of *Habrobracon hebetor* (Ode et al., 1997) and *Lariophagus distinguendus* (J. van den Assem, personal communication). In other parasitoids female behaviour does change post mating. For instance, *Melittobia acasta* females, once mated, can oviposit immediately and oviposition reaches a maximum in one or two days, but unmated females, when presented with a host, will sting it and host feed but not oviposit (van den Assem, 1976b; van den Assem et al., 1982a). *Cotesia glomerata* (Braconidae) virgin females lay consistently smaller clutches of eggs than mated females, both in the field and the laboratory (Tagawa, 1987). In *N. vitripennis* there is a clear behavioural switch after mating (Sect. 4.3.4): not only do females become unattracted to male pheromone secretions after mating (Ruther & Hammerl, 2014), they also become more active which is thought to reflect an increase in host-searching behaviour (King et al., 2000). However, despite being more active, mated females do not parasitise more hosts than do virgins. In addition to increasing (or decreasing) female longevity (Sect. 4.5.3), male ejaculate components have been shown to stimulate egg maturation, ovulation and oviposition in a range of insects (Avila et al., 2011). While there is evidence that male ejaculate components do influence female physiology and life history in *Nasonia* and other parasitoids, the seminal fluid components themselves and their mode of action are yet to be established.

4.6 Comparative Studies of Mating Behaviour

4.6.1 Mating Behaviour as a Source of Taxonomic Characters

The immense diversity of parasitoid wasps poses a major challenge to taxonomists. Sibling species abound, as do various degrees of incompatibility between field populations or laboratory strains, and in many cases, it is difficult to establish the species status of populations. Molecular techniques are valuable in untangling problems of relatedness (Molbo et al., 2002), but in some cases closely related species that are not sexually compatible cannot be distinguished genetically (e.g., *Lysiphlebus*: Starý et al., 2014). Investigation of courtship behaviour characteristics may provide an alternative, or complementary, approach to distinguish and identify cryptic species (Kazmer et al., 1996). Many courtship displays exhibit combinations of features that can be used for identification purposes (Gordh & DeBach, 1978; Joyce et al., 2010a, 2010b). Observations of courtship between species can also help test whether behaviourally different populations or putative species are reproductively isolated (either because they do not mate

or do not produce viable offspring after mating; Heimpel et al., 1997; König et al., 2019).

When looking for species-diagnostic characters, one can compare entire courtship displays (van den Assem et al., 1982a, 1982b; van den Assem & Gijswijt, 1989; Hunter et al., 1996). Records made with video equipment are useful as they allow repeated viewing and interspecific similarities and differences can be detected that might otherwise remain unnoticed. Besides the motor patterns of the appendages, it is the temporal organisation of displays (how they differ with respect to the order of appearance of components and to the number and lengths of intervals between components) that are particularly species diagnostic.

For example, *Muscidifurax* was long considered to be a monotypic genus, with *M. raptor*, a cosmopolitan parasitoid of the house-fly (*Musca domestica*), its only representative. However, Legner (1969) reported reproductive isolation of several of many strains in field-derived laboratory cultures, and five species were later recognised using morphological characters. These are, however, very variable and difficult to observe (Kogan & Legner, 1970). When males and females of the same and different strains were introduced, interspecific differences in display movements (patterns of antennal movements (Fig. 4.32) and in the duration of the intervals between successive display cycles) were readily observed, and the species were characterised behaviourally (van den Assem & Povel, 1973). There were also interspecific differences in males' treatment of either conspecific females or conspecific-like dummies, compared with their treatment of heterospecifics.

The case of *N. vitripennis* is very similar and illustrates the state of knowledge of the group as a whole. *Nasonia vitripennis* is probably the most intensively studied parasitoid species (Cousin, 1933; Whiting, 1967; Holmes, 1974; Burton-Chellew et al., 2008; Geuverink et al., 2009; Ruther et al., 2009, 2010; Werren & Loehlin, 2009; Lynch, 2015; Mair et al., 2017; Tappert et al., 2017; Lenschow et al., 2018; Mair & Ruther, 2019; Jones & Mallon, 2020; Pannebakker et al., 2020; Dalla Benetta et al., 2021; Jatsch & Ruther, 2021). The genus was once considered to be monotypic, its sole

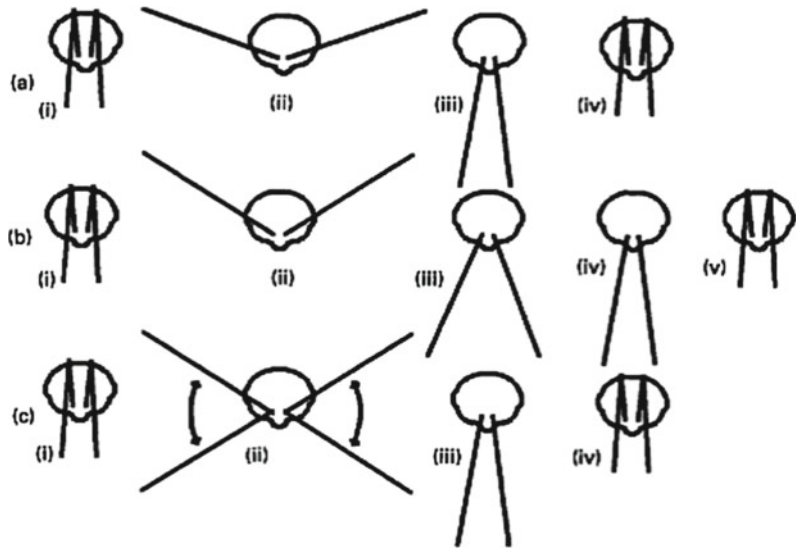

Fig. 4.32 Species-characteristic display movements with the antennae in three sibling species of *Muscidifurax* (Pteromalidae): **a** *M. raptor*, (i) start position, (ii) spreading the antennae, (iii) extreme low position, (iv) end position, **b** *M. zaraptor*, (i) start position, (ii) spreading the antennae, (iii) intermediate position during the slow downward movements, (iv) extreme low position, (v) end position; **c** *M. raptorellus*, (i) start position, (ii) waving episode, (iii) extreme low position, (iv) end position

representative being a cosmopolitan parasitoid of the pupae of cyclorrhaphan flies in a variety of habitats including manure, decaying carcasses and birds' nests (Werren, 1983). Through extensive collecting of fly puparia from birds' nests over a large area of the USA, three reproductively isolated strains were obtained (Darling & Werren, 1990). Members of different strains were sometimes collected from the same nest, or even from the same puparium. A morphological investigation led to the description of three species (Darling & Werren, 1990), and a fourth has now been identified (*N. oneida*, Raychoudhury et al., 2010) but simple diagnostic differences (except for wing length in males) could not be provided. As with *Muscidifurax*, studies of courtship behaviour revealed reliable diagnostic characters, namely differences both in the overall temporal organisation of displays and in the details of motor patterns (van den Assem & Werren, 1994; Dalla Benetta et al., 2021), as well as differences in where mating occurred (within the host or not; Sect. 4.3.1).

Mating behaviour was used, in conjunction with molecular techniques, to investigate reproductive incompatibility between strains of *Aphelinus asychis* (Aphelinidae), a parasitoid of aphids attacking wheat (Kazmer et al., 1996). Cultures were set up from wasps collected in the field in five Mediterranean and two Central Asian localities. *Aphelinus asychis* is released as a biological control agent against aphids, and the success of control programmes is likely to be influenced by the presence of reproductive incompatibility between released wasps. Initial investigations revealed that there were three reproductively incompatible groups among these cultures, and more detailed investigations of response to sex pheromone, courtship behaviour and sperm transfer were carried out to determine the basis of the incompatibility. Males responded normally to the sex pheromones (Sect. 4.3) of incompatible females and courted them actively. However, courtship by incompatible males did not usually induce receptivity by females, which did not stop moving or elevate their abdomens for copulation and, in most cases, there was no genital contact or sperm transfer. Reproductive incompatibility is apparently the result either of the failure of females to recognise males as potential mating partners, or of active rejection of males. Molecular investigations showed that the genetic distances between the three reproductively compatible groups were larger than between compatible groups.

Hunter et al. (1996) also used a combination of behavioural observations and molecular (allozyme) techniques to investigate whether three North American populations of *Eretmocerus eremicus* (Aphelinidae), parasitoids of whitefly (Hemiptera: Aleyrodidae), belong to a single species. There were no interpopulation differences in the kinds of courtship behaviour performed, the sequence of behavioural acts, the duration, or the frequency of components of courtship behaviour. There were, however, differences in behaviour when males and females from different populations were exposed to one another. Individuals from the Texas population did not copulate with individuals from Arizona or California, while California and Arizona individuals copulated (however, individuals from Arizona were relatively small, and in some cases wasps of differing sizes experienced difficulty in copulating) and there appear to be no post-copulatory reproductive barriers between these populations. Allozyme analysis (Sect. 5.3.3) supported the results from mating behaviour experiments: the Texas population was genetically distinct from the California and Arizona populations, and so Hunter et al. (1996) that the wasps studied belonged to two species.

Heimpel et al. (1997) used mating experiments in combination with life-history studies and mtDNA sequence analysis to distinguish between two populations of parasitoid identified as *Bracon hebetor* (now re-named *Habrobracon hebetor*). Parasitoids morphologically indistinguishable from *B. hebetor* were released in Barbados from 1970 to 1975 to control *Helicoverpa* (= *Heliothis*) spp. (Lepidoptera: Noctuidae). In the USA, *H. hebetor* is known as a parasitoid of phycitine moths (Lepidoptera: Pyralidae). The differences in host use suggested that the Barbados parasitoids and the American parasitoids might belong to a different species.

Pairs of males and females from different 'strains' were placed together for 48 h to allow mating, and a host was provided for oviposition. On dissection, the spermathecae of the females were found to be empty, and all progeny reared from the hosts were male (arising from unfertilised eggs). The two 'strains' are thus reproductively isolated, and subsequent behavioural observations showed that no 'inter-strain' mating occurred: in one case a male produced a courtship display, but copulation was never observed. Immediately after wasps from different 'strains' had been observed, each individual of the pair was observed with a member of the same 'strain': in 30 out of 34 cases both individuals mated with a member of their own 'strain'. Genetic analysis showed that divergence between the 'strains' is consistent with interspecific differences. Heimpel et al. (1997) therefore concluded that the parasitoids from Barbados belong to a species reproductively distinct from *H. hebetor*.

4.6.2 Mating Behaviour and Phylogenetics

The aims of comparative studies of the mating behaviour of parasitoids and predators go beyond rendering assistance in problems of taxonomic identification. Such studies, if they use the appropriate analytical techniques, can also enable us to estimate a how behaviour evolved; i.e., changed during phylogeny. Parasitoids and predators, because of their high biological diversity, provide excellent material for comparative work (Goodpasture, 1975; Boulton & Heimpel, 2018; Böttinger & Stökl, 2020). Behavioural traits can be mapped to morphological or molecular phylogenies and ancestral-state reconstructions carried out to estimate what the most likely sequence of evolutionary events was that led to the current diversity of behaviour. Using ancestral-state reconstructions, Klopfstein et al. (2010) showed that a male courtship-related trait, antennal coiling (which is assessed in museum specimens and acts as a proxy for antennal movement during courtship), has evolved independently more than once in a subfamily of Ichneumonid wasps, but once this trait is lost it does not re-evolve. However, observed variation does not always relate closely to phylogeny (Böttinger & Stökl, 2020).

Other comparative methods provide tests of correlated evolution, which can show whether the evolution of two or more traits was independent (i.e., Ridley, 1993; see also Brooks & McLennan, 1991; Harvey & Pagel, 1991; Mayhew & Pen, 2002; Sects. 1.2.3 and 5.3.4). The abovementioned study by Klopfstein et al. (2010) also found evidence for correlated evolution between the behavioural (antennal coiling) and morphological (sexually dimorphic antennae) traits; these traits did not evolve independently of one another, suggesting that courtship behaviour and morphology co-evolve in these wasps.

Differences among parasitoid wasp taxa in the courting male's orientation with respect to the female can be viewed as indicators of a phylogenetic transformation series (a series of inferred trait changes that occur over evolutionary time; Hölldobler & Wilson, 1983). In some groups, the male adopts the same position in courtship as in copulation; i.e., to the rear (*Trichogramma evanescens:* Hase, 1925; *Brachymeria intermedia:* Leonard & Ringo, 1978; *Spalangia nigra :* Parker and Thompson, 1925; *Spalangia endius:* van den Assem, 1986). In most parasitoid wasps, however, positions during courtship and copulation are distinctly different: males court away from the mating position, and mostly perform either on top of the female or near to her on the substratum (Gordh & DeBach, 1978; Bryan, 1980; Grissell & Goodpasture, 1981; Orr & Borden, 1983). A hypothesised phylogenetic sequence of positional changes is summarised in Fig. 4.6. While it appears obvious that the latter (courtship and copulation postures different) is the ancestral state which has given rise to the former (the same posture for courtship and copulation), this remains to be explicitly tested (although see Rhoades, 2015, for a reconstruction of antennal position during courtship in the genus *Aphelinus*), and in some cases hypothesised models of evolution have not held up to rigorous phylogenetic testing (Field et al., 2020).

If the hypothesised sequence of evolutionary events is robust, the tendency towards a more anterior courtship position is apparent in several families and subfamilies and may represent a response to a general selection pressure, perhaps more efficient communication at the front due to the presence of more diverse or more dense sense organs at the anterior ends of both sexes (van den Assem & Jachmann, 1982).

In a comparative study of mating behaviour, it is important to possess a phylogeny of the taxa involved; one may already exist in the literature, willing taxonomists may provide a provisional tree, and generating a molecular phylogeny based on available genetic sequence data is now relatively straightforward, as much data is available in the GenBank database (NCBI: National Centre for Biotechnology Information; Benson et al., 2012). Mating behaviour traits need to constitute an independent data set, so the phylogeny needs to have been constructed using other (molecular and morphological) characters. By 'mapping' mating behaviour traits onto the tree, inferences can be drawn as to when a particular trait has evolved and whether it has arisen independently in the evolution of the insect group or is ancestral. Comparative studies of parasitoid mating are further discussed in Sect. 5.3.4.

4.7 Conclusion

The study of insect mating behaviour continues to provide very valuable insights into evolutionary processes and relationships. Despite the increasingly reductionist nature of the biological sciences, the study of insect behaviour, within a sound evolutionary framework, has major interpretative and heuristic value. This can be of importance both to the conceptual understanding of behavioural evolution and to assessing the consequences of such evolution for practical applications in biological pest control and integrated pest management (Sect. 4.1.1; Tappert et al., 2017).

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Mating Systems

5

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5.1 Introduction

The term 'mating system' is used to describe how males and females obtain mates in a population (Emlen & Oring, 1977; Thornhill & Alcock, 1982; Davies, 1991; Brown et al., 1997; Shuker & Simmons, 2014). A particular mating system may be characterised by the events surrounding pair formation, courtship, copulation and the post-copulatory events (Brown et al., 1997). Individual males and females engage in reproductive behaviours that maximise their own fitness, frequently to the detriment of their mates (sexual conflict; Chapman et al., 2003). Evolutionary biologists have come to regard events surrounding mating as a set of intra-sexual and inter-sexual 'battles' which reflect the sometimes mutual, sometimes differing, reproductive interests of males and females (e.g., Davies, 1991; Brown et al., 1997; Choe & Crespi, 1997; Alonzo & Warner, 2000; Chapman et al., 2003; Arnqvist & Rowe, 2005; Lessells, 2012; Kokko et al., 2014).

The mating systems of insect natural enemies are diverse and, despite the large number of studies carried out to date, there is still ample scope for further fruitful investigation. There are two main reasons why insect natural enemy mating systems have been investigated: (1) to understand the evolution of mating systems themselves, and (2) to understand the evolution of sex allocation decisions (which is likely to be greatly influenced by details of the mating system; Boulton et al., 2015). An additional reason, which has received relatively little empirical attention, is an improved understanding of the conditions required for successful establishment of a natural enemy population during the implementation of biological programs (e.g., Hopper & Roush, 1993; Meunier & Bernstein, 2002; Leung et al., 2019; Segoli et al., 2023).

Although these reasons are interrelated, something of a taxonomic dichotomy has developed in the study of mating systems. Workers on parasitoids (mostly Hymenoptera, which have a high degree of control over sex allocation due to haplodiploidy; Sect. 3.3) have tended to investigate mating systems as a backdrop to sex ratio research (Boulton et al., 2015). Workers on predatory insects (many of which

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may be constrained in their control of sex allocation) have tended to ask questions about mating systems per se, including considerations of sexual conflict. Of course, there are welcome exceptions to this 'dichotomy'—in particular, the idea that parasitoids can be useful models to understand mating system evolution continues to gain traction (i.e., Boulton et al., 2015; Liu et al., 2017; Abe, 2019; Böttinger & Stökl, 2020)—but it will nevertheless be reflected in the structure of this chapter.

We begin by illustrating the diversity of mating systems among insect natural enemies (Sect. 5.2). We next discuss general approaches to their study (Sect. 5.3) and then review investigations of parasitoid biology that have examined one or more components of these mating systems (Sect. 5.4). Section 5.4 is organised by 'issue', with methodology highlighted within each subsection. In Sect. 5.5 we consider the mating systems of predatory, rather than parasitic, natural enemies.

In this chapter we take a more 'functional' approach than that taken in the companion chapter on *Mating Behaviour* (Chap. 4). Rather than the fine details of the behavioural mechanisms involved, we are principally concerned with the causes and consequences of the mating patterns within a population (the 'What is it for?' of Tinbergen's four questions, discussed in Chap. 4). However, it will become clear that there is great overlap and productive interchange between these research areas.

5.2 Mating Systems Theory

The majority of work aimed at understanding the evolution of insect mating systems takes Bateman's principle as its central tenet (Bateman, 1948; Lehtonen, 2022). In short, this posits that females are the limiting sex since they cannot increase their fitness via multiple mating in the same manner as can males (females are the limiting sex because they produce far fewer eggs than males produce sperm). In recent years the broad applicability of this principle has been called into question (Tang-Martinez, 2016; Morimoto, 2020; Hoquet, 2020a, b; Lehtonen, 2022) but in general males tend to compete for access to females, and females tend to be the choosier sex in regard to mating decisions. The combination of this basic evolutionary principle with the fact that ecological variables and constraints determine the spatial and temporal availability of females results in the evolutionary conditions that produce the mating system of an organism (Bradbury & Vehrencamp, 1977; Emlen & Oring, 1977; Andersson, 1994; Kokko et al., 2014). This ecologically based view of mating systems has been particularly fruitful in understanding the evolution of diverse insect reproductive traits (e.g., Thornhill & Alcock, 1982; Choe & Crespi, 1997; Shuker & Simmons, 2014).

As previous authors have noted, there are various ways of classifying animal mating systems (e.g., Emlen & Oring, 1977; Thornhill & Alcock, 1982; Davies, 1991; Reynolds, 1996; Brown et al., 1997; Godfray & Cook, 1997; Kokko et al., 2014). Traditionally, mating systems were classified according to sex differences in parental care, with the sex with smaller gamete size and the highest rate of reproduction (due to faster rates of gametogenesis and/or lower investment in the form of parental care) being the one most likely to compete for members of the opposite sex (Baylis, 1978, 1981; Reynolds, 1996; Lessells, 2012; Wong et al., 2013). However, it has become increasingly apparent that considering mating systems in this way provides only a superficial view of their evolution (Boulton et al., 2018a, b). Rather, the focus of mating systems studies has shifted to the number of mating partners for females or males. Polyandry and polygyny refer to multiple mating by females and males respectively, and monandry and monogyny refer to mating with a single partner.

In this chapter, we define the mating system as the way a group of organisms evolves to maximise (individual) fitness by optimising reproductive opportunities. We follow the mating classification scheme described by Brown et al., (1997; Fig. 5.1) which considers how environmental and phylogenetic factors contribute to reciprocal selection between the sexes, the outcome being dynamic and evolving mating systems.

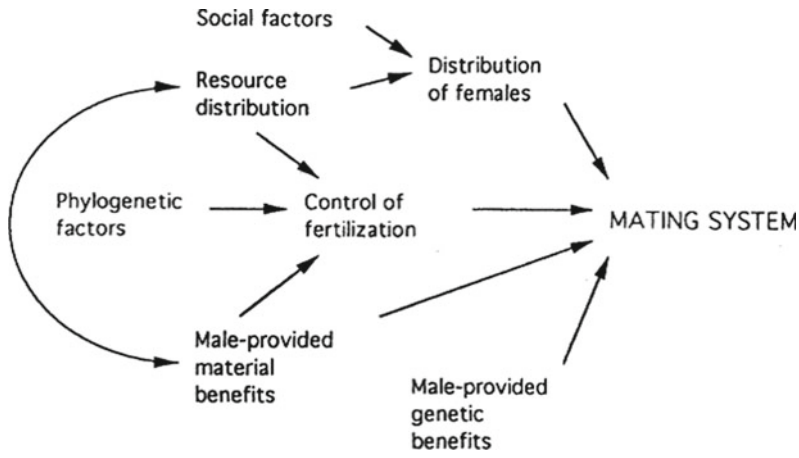

Fig. 5.1 The main factors influencing animal mating systems. 'Phylogenetic factors' refers to the historical presence or absence of adaptations related to the control of fertilisation in various lineages. *Source* Brown et al. (1997). Reproduced by permission of Cambridge University Press

As noted above, studies of the mating systems of parasitic hymenopterans and predatory insects have focused on different issues and this is also reflected in the classification schemes for mating systems in these two groups of organisms. Classifications of parasitoid mating systems are for the most part much simpler than those developed for predators. Parasitoid mating systems are primarily concerned with the issue of whether mating is restricted and local (mating opportunities are primarily in the natal area) or random and panmictic (mating opportunities extend throughout the population). Here we follow the mating classification adopted by Godfray and Cook (1997) which focuses on the site where mating occurs (see also Table 5.1). With few exceptions (e.g., Hamerski & Hall, 1989; Hardy & Blackburn, 1991; Field & Keller, 1993; Allen et al., 1994; Fischer, 2005; Leonard & Boake, 2008; King & Kuban, 2012; Pan et al., 2018) prolonged post-copulatory associations (e.g., parental care or mate guarding) are absent in the parasitic Hymenoptera although brief post-copulatory courtship displays of some species act as mate guarding *in absentia* (Chap. 4; Boulton et al., 2015).

Given the diversity of life-history and feeding strategies in non-parasitoid insect taxa, it is not surprising that the ecological constraints on mating systems are many and varied and, consequently, there is a broad range of mating system types (see Thornhill & Alcock, 1982; Shuker & Simmons, 2014, for reviews). The starting point for classifying mating systems is the propensity of each sex to mate during its reproductive lifetime. Consequently, mating systems are monogamous (both sexes mating with a single partner), polygynous, polyandrous or polygamous (both sexes mating with multiple partners) (Krebs & Davies, 1993; Kokko et al., 2014).

5.3 Methods of Studying Mating Systems

In this section we provide an overview of general approaches to studying the mating systems of insect natural enemies. Examples of applications of these methods in the study of particular mating systems are given in the following sections. Empirical approaches can be classified as 'behavioural', 'genetic' and 'comparative'. Theoretical models can also be employed to develop a framework of predictions that form a conceptual context for empirical work. The application of two or more of these approaches in concert is likely to prove synergistic.

Table 5.1 Summary of predicted sex ratio optima under different mating systems

Mating structure		
(a) Local mating	(b) Within patch	Sex ratio
Full	Sib-mating biased	Female bias greater than Hamilton's prediction (7)
	Random	Hamilton's prediction: Sex ratio sensitive to foundress number, more female biased at lower foundress numbers (2,3,4,5,6,7,9)
	Sib-mating avoided	Less female bias than under random mating (8)
Partial	Sib-mating biased	Female bias decreased relative to equivalent conditions under fully local mating, and increased relative to partial local mating with random on-patch mating (6) (see also 10,11)
	Random	Female bias decreased compared to Hamilton's prediction (4,5,6,7,9) Different sex-ratio optima for sib-mated and non-sib-mated mothers (10)
	Sib-mating avoided	Not formally studied (but see 10): we would expect little or no female bias
None	None	Sex ratio equality (1)

Mating system is represented by two components: (a) the degree of local mating on the patch (Full = all mating is local, Partial = some mating is local and some non-local, None = panmixis), and (b) where there is at least some local mating, the mating structure within the patch itself. Sex ratio predicted under each mating system is briefly summarised relative to Hamilton's (1967) initial model of sex ratio under strict local mate competition with random mating within each patch. Modelling studies considering further influences on sex ratio optima under local mate competition are summarized in Godfray (1994, p. 167), Hardy (1994a, 2002a), Hardy et al. (1998), and West (2009); see also Kathuria et al. (1999), Gardner and Hardy (2020), Gardner (2023) and Lehtonen et al. (2023). References: (1) Düsing (1884), Fisher (1930), (2) Hamilton (1967), (3) Werren (1984), (4) Taylor and Bulmer (1980), (5) Frank (1986), (6) Nunney and Luck (1988), (7) Werren and Simbolotti (1989), (8) Stubblefield and Seger (1990), (9) Ikawa et al. (1993), (10) Greeff (1996), (11) Shuker et al. (2005) (see also Denver & Taylor, 1995; Greeff & Taylor, 1997; Martel et al., 2016). *Source* Modified from Hardy (1994a), who also considers the influence of host quality

5.3.1 Theory and Modelling Approaches

The various modelling approaches used by evolutionary biologists can be classified as 'genetic' and 'non-genetic'. Genetic models are those that explicitly model a given behaviour, examining the conditions that favour the spread of a rare allele for a trait through the population. Antolin (1993) provides a summary of genetic models of sex ratio in subdivided populations. Non-genetic or phenotypic models examine a behavioural trait in terms of its contribution to an individual's fitness. Much behavioural ecology is based on non-genetic or phenotypic models, despite the recognition that population genetics underlies behavioural ecology (Grafen, 1991). Grafen (1991) provides a detailed discussion of phenotypic modelling and concludes that, although it may occasionally be incorrect, it is generally a well justified approach for studying the selective forces that influence character evolution. Phenotypic models of mating systems may focus on either the population or the individual. Examples of population-based phenotypic models of mating systems include the group of related sex allocation models along the continuum from local mate competition to random panmictic mating (Table 5.1). Hardy (1994a), Godfray (1994), West (2009) and the sections below provide discussion and examples of mating systems that are intermediate.

Phenotypic models that describe mating systems in organisms practising parental care tend to focus on individuals. An important consideration when modelling mating systems is that individuals of one sex commonly compete amongst themselves for mating opportunities with

members of the opposite sex. For a particular individual, the best solution to such problems is likely to depend on the behaviour of its competitor(s), but competitor behaviour will also be influenced by the behaviour of the individual. Game theoretic (or ESS) models (Sect. 1.2.2) are frequently used to explore such situations. Within a population, a variety of different tactics to obtain copulations may coexist. At least three possibilities exist: (1) alternative strategies where the difference in tactics used by individuals has a genetic basis; (2) mixed strategies, where each individual exhibits a probabilistic mix of tactics; and (3) conditional strategies, where the tactic employed by a given individual is dependent on the status of that individual (Gross, 1996). While there are limited numbers of examples of populations with a mixed strategy, examples of male conditional strategies abound (Davies, 1991; Gross, 1996; Alonzo & Warner, 2000). In several mating systems such alternatives frequently exist, e.g., 'mate guarding' (attempting to exclude competitors: Fig. 5.2) or 'mate sneaking' (obtaining copulations with females guarded by other males). Which alternative is adopted may simply reflect the limited capabilities of some individuals. For example, if successful mate guarding generates high fitness returns but males vary in mate guarding ability (e.g., dependent on body size), inferior males attempting to mate guard fare very poorly indeed but gain intermediate fitness by adopting the tactic of sneaking mating opportunities with females guarded by superior males. Thus, inferior males may 'make the best of a bad job'. It may, however, be possible that alternative tactics generate equal fitness returns either due to frequency-dependent selection (e.g., Hamilton, 1979; Dominey, 1984; Cook et al., 1997; Kokko et al., 2007) or because alternative tactics are advantageous after mating, when competition over fertilisation occurs (post-copulatory sexual selection; Boulton et al., 2015).

Alternative mating tactics have not been widely investigated in natural enemies and few examples have been uncovered so far. The best described example comes from the encyrtid *Ooencyrtus kuvanae*, where three male strategies have been reported (although see Boulton et al., 2015). The 'mate-at-once' strategy involves mating with the first receptive female encountered; 'harem gathering and guarding' involves males pheromonally tagging females, rendering them unattractive to competitors, then returning to mate. Finally, 'sneaking' involves a second male copulating with a female who is engaged in post-copulatory courtship with her previous mate (Ablard et al., 2013, 2014). This example represents a mixed strategy, as 60% of males engage in both harem gathering and mating at once, with the former providing the greatest fitness gains. It is not yet clear whether sneaking is also a plastic trait, and whether any or all of these strategies are condition dependent or evolutionarily stable remains to be tested. Considering this example under the framework provided by Alonzo and Warner (2000), which uses models of dynamic state variable games, would be a valuable way of exploring mating system evolution in this system, and in other examples from natural enemies as they are discovered.

5.3.2 Behavioural Studies

Relatively simple observations of behaviour, whether in an experimental or natural setting, can provide an enormous amount of information about components of mating systems. Behavioural studies have been the backbone of mating system studies in the past and are likely to continue to play an important role. The methodology involved in behavioural studies does not need to be complex, but there are ongoing advances in methods of recording and analysing behavioural data (e.g., Haccou & Meelis, 1992; Fauvergue et al., 1999; Sect. 4.2 and Chap. 9). General introductions to methodology of behavioural studies are given by Martin and Bateson (1996) and Bateson and Martin (2021). In general, mating system studies start with the careful observation of marked individuals throughout a reproductive period/season and aim to document the nature and frequency of mate encounters, the proportion of mating encounters that males and females accept, whether any factors alter

Fig. 5.2 Mate guarding in the damselfly *Ceriagrion tenellum*. After copulation the male remains attached to the female's prothorax, with his abdominal cerci, and accompanies her during oviposition. By remaining attached in this way he ensures that other males cannot mate with her. The pair separates once the female has laid her current clutch of eggs and she therefore no longer provides an immediate opportunity for male reproductive success. *Photograph* M. Siva-Jothy

likelihood of copulation occurring, and how much variance in mating (and insemination) success exists. Additional parameters to measure include any behavioural or physiological traits that determine the operational sex ratio (OSR, the ratio of sexually active males to sexually active females; for discussions of OSR see Kvarnemo & Ahnestjö, 2002; Kokko et al., 2012). Such studies can be accompanied by investigation aimed at understanding the pattern of sperm precedence (Sect. 4.5.2) which, when combined with an understanding of the pattern of egg laying, enables the conversion of measures of mating success to reproductive success. Traditionally, measurements of female behaviour and physiology tend to concentrate on factors that determine female reproductive value and the female role in determining OSRs. Although females are increasingly considered as more than passive resources to be defended by active males in mating systems research (Boulton et al., 2018a, b), the combination of these more traditional approaches has provided the basis for describing and understanding insect mating systems. The ultimate determinants of the mating system (inevitably ecological variables) can then be narrowed down and identified experimentally.

This mainly behavioural approach has been used recently in powerful combination with modern empirical approaches, such as genetic techniques for assessing levels of inbreeding and outbreeding and identifying paternity (Sect. 5.3.3) and with data on further species, making use of recent advances in comparative methods (Sect. 5.3.4). As noted above, there has long been a productive interchange between behavioural studies and theory-driven hypotheses.

5.3.3 Genetic Studies

Insect natural enemies are generally small, and the reproductive behaviours of individuals are usually difficult to observe, especially if mating takes place in localities other than the natal site or at the host. Advances in the methodology of genetic studies in recent years offer a solution because important elements of mating systems can be estimated without observing mating or parental behaviour (Miño et al., 2011). Despite these developments, and their applications to mating systems of animals such as birds, mammals and social insects (e.g., Honeycut et al., 1991; Davies, 1992; Fjerdingstad & Boomsma, 1998; Hughes et al., 2008a, b; Miño et al., 2011), genetic studies have taken longer to become established tools for investigating insect natural enemy mating systems, but there has been considerable growth in recent years (e.g., Allen et al., 1994; Molbo & Parker, 1996; Antolin, 1999; Antolin et al., 2003; Burton-Chellew et al., 2008; de Boer et al., 2015; Abe & Pannebakker, 2017; Sepúlveda et al., 2017; Garba et al., 2019; Collet et al., 2020; Abe et al., 2021; see Macdonald & Loxdale, 2004, for an overview).

Some studies of insect natural enemies have used genetic techniques to characterise mating systems according to how different traits, for instance dispersal, host choice, mate guarding and body size, correlate with key components of the mating system, such as population structure, sex ratio and fitness outcomes (e.g., Kazmer & Luck, 1991; Allen et al., 1994; Molbo & Parker, 1996; Abe et al., 2021). Other studies have focused on genetics as a force driving the evolution of mating systems (e.g., selecting for traits such as dispersal that reduce the risk of inbreeding depression Antolin & Strand, 1992; Cook & Crozier, 1995; Godfray & Cook, 1997; Antolin, 1999; Antolin et al., 2003; Ruf et al., 2011; Sepúlveda et al., 2017; Abe & Pannebakker, 2017; Garba et al., 2019; Collet et al., 2020; see de Boer et al., 2021, for a recent meta-analysis of inbreeding in diploid animals).

The influence of mating systems on genetic variation

The basic procedure is to obtain estimates of genetic variability at polymorphic loci and compare these to expectations from the Hardy–Weinberg theorem under population-wide random mating (panmixis). The Hardy–Weinberg equilibrium is the ratio of genotypes that evolve in infinite populations (>1000 individuals) when mating is random and neither natural selection nor genetic drift are operating (e.g., Ridley, 1993a). Deviations from Hardy–Weinberg expectation imply that mating is non-random throughout the population, that the population is finite and/or that natural selection is operating. A major reason for non-random mating is that populations consist of isolated or partially isolated sub-populations (or demes).

Various techniques can be used to assess variation in DNA or its products (Sect. 3.2; an entomological introduction is provided by Cook, 1996, and see Kazmer, 1990; Hadrys & Siva-Jothy, 1994; Hoy, 1994; Scott & Williams, 1994; Behura, 2006). An established, economical, and powerful method involves screening the electrophoretic mobility of enzymes (DNA products) (Sects. 3.2.2 and 6.3.11).

Although enzyme markers can be used to study population structure and the parentage or relatedness of individuals, they are often relatively invariant compared to DNA and, since they can be expressed at different points in the life history of an insect, they can be unreliable if used to compare adults and early instars, especially in holometabolous insects. Furthermore, variation in allozyme frequencies may themselves reflect selection on traits other than those reflecting population mating structure.

The range of molecular and associated analytical techniques for the direct assessment of DNA variation has, since the publication of the first edition of this book, expanded dramatically. Examples include random amplified polymorphic DNA [RAPDs], DNA fingerprints [using RFLP;

restriction length polymorphism], DNA microsatellites and amplified fragment length polymorphisms [AFLPs] (Sect. 3.2; and further reviews in Queller et al., 1993; Avise, 1994; Cook, 1996; Mueller & LaReesa Wolfenberger, 1999). RAPD and AFLP amplify certain regions in the DNA and allow testing of relatedness and population structure based on the number and size of the fragments that are cleaved and amplified. These techniques do not require time-consuming primer design and so can be done rapidly using 'off-the-shelf' kits. AFLP in particular requires no prior knowledge of the organism's genome. Despite some impressive successes resulting from the application of RAPD to insect mating systems (e.g., Hadrys et al., 1992, 1993), the technique has largely fallen out of use. This is perhaps due to the limited scope of information available (nucleotide sequences are unknown and it is generally not possible to assign individuals as hetero- or homozygous; Allan & Max, 2010) as well as problems with cross-contamination from other genomes compared to recently developed techniques.

The most commonly used techniques for assessing genetic variation involve amplification of DNA microsatellites (MSATs) which are repetitive dinucleotide sequences in which the number of repeats at a locus is variable. MSATs are routinely used to identify individuals, assign kinship and assess differentiation between populations and sub-populations (Allan & Max, 2010). Microsatellites have advantages over other genetic markers in that they are highly polymorphic single loci, thereby providing much information (examples of the application of microsatellites to mating system research are provided by Estoup et al., 1994; Molbo et al., 2002, see also Abe et al., 2021; Liu et al., 2023). The technique is PCR based, and so one can use minute quantities of DNA, and the results can be rapidly and inexpensively visualized using fluorescent emission of labelled primers (rather than more time- and resource-consuming gel electrophoresis). Multiple variable loci occur but each locus can be studied independently, which aids interpretation and allows the use of existing computer programs for data analysis (e.g., Goodnight & Queller, 1999; Wang, 2009; Jones & Wang, 2010). The technique has one time-consuming step: the development of primers for individual repetitive loci for the species concerned. However, more and more primers are becoming available and expertise in primer development is now common.

Perhaps the most important advances have been in the development of next-generation sequencing techniques (SOLiD, Illumina and Pyro sequencing), which has allowed whole genomes to be sequenced rapidly at very low cost (around $0.10 for 1 million base pairs, compared to $1000 for Sanger sequencing). Next-generation sequencing has meant genomes for non-model species can be assembled cost-effectively and that single nucleotide polymorphisms (SNPs) can be easily identified and used to investigate population structure (see Helyar et al., 2011, for a review). Although MSATs tend to have higher allelic diversity per locus and have the advantage of being selectively neutral, SNPs can be highly informative for population structuring. When combined with the relative ease and speed with which SNP markers are developed and screened, their value for studying the genetic population structure of small and understudied non-model species, such as many natural enemies, is clear (de Boer et al., 2015).

The influence of genetics on mating systems

There are several ways in which genetic systems possessed by insect natural enemies can influence the evolution of their mating systems. Here we discuss the ability of females to reproduce without mating and the genetic disadvantages of mating between close relatives. Individuals of one sex may also adopt an alternative mating tactic, which may have a genetic basis (e.g., Gross, 1996; Neff & Svensson, 2013; Sect. 5.3.1).

Virgin reproduction

Many insect species have a chromosomal, or heterogametic, sex determination mechanism (both sexes are diploid) (e.g., Cook, 2002; Blackmon et al., 2017). In such species, the sex chromosomes inherited at the time of fertilisation dictate whether an individual develops as a male

or as a female; one sex is heterogametic and the other homogametic. Aside from possibly constraining the production of biased sex ratios (Hamilton, 1979; Bull & Charnov, 1988; Hardy, 2002b; West & Sheldon, 2002), heterogamety requires that eggs must be fertilised before they can develop and thus females must mate (or self-fertilise) to reproduce. In contrast, other insect species, including many parasitoid wasps, are arrhenotokous (e.g., Cook, 2002; Blackmon et al., 2017). Under arrhenotoky, fertilised (diploid) eggs generally develop into female offspring and unfertilised (haploid) eggs develop into males (Sect. 3.3). Once mated, females store sperm (in the spermatheca) and potentially control the sex of offspring by regulating the fertilisation of eggs (hence the enormous interest in parasitoid sex ratios). Virgin females are also able to reproduce but are constrained to produce male offspring only. Empirical observations indicate that, with some exceptions, virgin parasitoids make ready use of this ability (e.g., Godfray & Hardy, 1993; Ode et al., 1997).

Virginity has been studied in relation to mating systems using an ESS model (Godfray, 1990; Sect. 5.3.1) and in relation to clutch size and developmental mortality under strict local mating (Sect. 5.4.2) using a static optimality model (Heimpel, 1994; Sect. 5.4.7; see also Kokko & Mappes, 2005, for a more general theoretical perspective). Tests of model predictions require estimates of the prevalence of virginity and there are several methods available (Godfray, 1988, 1990; Hardy & Godfray, 1990; Godfray & Hardy, 1993; Hardy & Cook, 1995; Ode et al., 1997; Hardy et al., 1998; West et al., 1998). A direct method is to dissect females that are foraging for oviposition opportunities, or those that have dispersed from the natal site in species with strict local mating, and examine the contents of their spermathecae: females without sperm are either virgin or have been mated but become sperm depleted. Alternatively, females can be provided with hosts suitable for daughter production, and the sex of their progeny examined. Molecular methods can be integrally used to estimate actual *versus* realized paternity, as well as patterns of sib-mating, by genotyping spermathecal contents and offspring respectively (Fernández-Escudero et al., 2002). Indirect methods can be used for gregarious species in which sex ratio optima are likely to be female biased or unbiased. Broods containing only males are likely to have been produced by virgin mothers and the proportion of all-male broods serves as an estimate of the proportion of virginity. Similarly, in species with strict local mating, females that develop in all-female broods (e.g., due to developmental mortality of males) will remain virgin, and the proportion of such females observed can be used as an estimator of the proportion of virginity in the population.

Empirical work by King (2002) found that female *Spalangia endius*, a parasitoid with a local mating population structure, produced a greater proportion of sons among their offspring in response to the presence of another mated female conspecific, but not when the conspecific was a virgin, a finding consistent with predictions of local mate competition theory (Sect. 5.4.2).

Some parasitoid species are thelytokous: males are not produced and females arise from unfertilised eggs that become diploid by fusion of meiotic products (e.g., Luck et al., 1993; Cook, 1993a; Schneider et al., 2002; Belshaw & Quicke, 2003; see Sect. 3.3.2 for more detail). Obviously, thelytokous species do not have mating systems as mating does not occur. However, some species have thelytokous and sexual members (e.g., Schneider et al., 2002; Sandrock & Vorburger, 2011). Thelytoky is sometimes caused by microbial (*Wolbachia*) infection in some parasitoids and can be cured by antibiotic or high-temperature treatment (Stouthamer et al., 1992a, 2002; Arakaki et al., 2000; Pannebakker et al., 2004). In *Trichogramma* spp. (egg parasitoids), most infected populations consist of infected and uninfected individuals (Gonçalves et al., 2006) but in all other documented cases infections have gone to fixation in the population (Stouthamer, 1997). In some parasitoid species, the males produced by cured females have often lost the full capacity to perform mating behaviour (Stouthamer, 1997). Further, thelytoky may be more likely to arise in species with high levels of

inbreeding, as it circumvents problems associated with producing highly female-biased and precise sex ratios (Cornell, 1988; Hardy, 1992; Cook, 1993a; Hardy et al., 1998; Khidr et al., 2013; Wilkinson et al., 2016, Sect. 5.4.5); this constitutes a further potential influence of mating systems on genetics.

Inbreeding Depression and Sex Determination in the Hymenoptera

Unlike diplo-diploid species, sex in the Hymenoptera is not determined by sex chromosomes or the ratio of sex chromosomes to autosomes. Instead, Hymenoptera are haplodiploid, with males developing from unfertilised eggs and females developing from fertilised eggs (e.g., Cook, 2002). The discovery of diploid males in *Habrobracon hebetor* nearly 100 years ago (Whiting & Whiting, 1925) prompted four models of sex determination in the Hymenoptera: single-locus complementary sex determination (CSD), multiple-locus CSD, genic balance, and genomic imprinting. Of these, single-locus CSD is thought to have the biggest impact on mating systems because its effects are exacerbated during inbreeding (sustained inbreeding of species with multiple-locus CSD is also expected to generate diploid males).

Diploid males have been found across the Hymenoptera (Cook, 1993a; Duchateau et al., 1994; Carvalho et al., 1995; Holloway et al., 1999), in the Apoidea (bees), Vespoidea ('social' wasps), Tenthredinoidea (sawflies), Ichneumonoidea, Chalcidoidea, and the Cynipoidea (van Wilgenburg et al., 2006, and Heimpel & de Boer, 2008, provide more comprehensive reviews). Various techniques have been used to detect diploid males, including karyotyping, flow cytometry, visible genetic markers, allozymes and molecular markers, and morphological characters (Cook, 1993a; van Wilgenburg et al., 2006; Asplen et al., 2009; Weis et al., 2017). All of these techniques are dependent on diploid males developing to the point at which they can be sexed (typically as pupae or adults). In many of the species in which diploid males are known, their viability is similar to that of haploid males and diploid females. Yet, diploid male production may be much more prevalent than the number of known cases suggests; if diploid males are all inviable, diploid male production can go unnoticed. In this case, even if 'ploidy' can be established for eggs or larvae it may be very difficult, if not impossible, to assign sex to a diploid individual. One possible exception would be the identification of a sex-specific protein or imaginal disk tissue present early in development. Statistical comparison of survivorship of eggs from inbred and outbred females can provide support for the presence or absence of CSD as well as the number of loci involved (Weis et al., 2017).

Many of the species known to produce diploid males appear to follow the single-locus CSD model (reviewed in Cook, 1993a; van Wilgenburg et al., 2006), where sex is determined at a single locus (Whiting, 1939, 1943). Under single-locus CSD, individuals that are heterozygous (diploid) at the sex locus develop into females, whereas individuals that are either hemizygous (haploid) or homozygous (diploid) at the sex locus develop into males. An important prediction of the single-locus CSD model is that diploid male production should increase with inbreeding (inbreeding increases levels of homozygosity at all loci including the sex locus). The impact that single-locus CSD has on a population mating system depends on the interaction between the amount of inbreeding, the number of sex alleles in the population, the number of times a female will mate, and whether diploid males are viable, able to disperse and can transfer sperm to females (Cook & Crozier, 1995; Winkert et al., 2019). Species possessing single-locus CSD may avoid high rates of diploid male production by outbreeding; such appears to be the case with *Habrobracon* (= *Bracon*) *hebetor* (Antolin & Strand, 1992; Ode et al., 1995; Antolin et al., 2003; see also Garba et al., 2019). Outbreeding coupled with high sex allele diversity within populations can reduce the genetic load associated with single-locus CSD. Allelic diversity is expected to be maintained by strong frequency-dependent selection. The number of sex alleles in a population has been estimated using matched matings (e.g., Whiting, 1943;

Heimpel et al., 1999; Zhou et al., 2006; Weis et al., 2017) as well as maximum-likelihood approaches (e.g., Owen & Packer, 1994) and, most recently, a whole genome approach combined with flow cytometry to extrapolate from population levels of kin mating and diploid male production (de Boer et al., 2015). Estimates in natural populations appear to be high enough to make the production of diploid males rare. Finally, there may be selection for avoidance of mating with close relatives (e.g., Ode et al., 1995; Metzger et al., 2010; Collet et al., 2020) or for polyandry (e.g., Page, 1980). In species where females mate only once, females that mate with viable diploid males are constrained to produce only sons (e.g., Heimpel et al., 1999); multiple mating can increase the chance that females can produce daughters, assuming that multiple mating increases the likelihood of mating with a haploid male (see Boulton et al., 2015, for a review). Such considerations may be important when mass-rearing parasitoids for biological control programmes. Initial sampling procedures should involve obtaining naturally high allelic diversity in the founder culture of species with CSD, and measures such as maintaining large cultures or parallel smaller subcultures are recommended to prevent loss of allelic diversity during culturing (Stouthamer et al., 1992b; Cook, 1993b).

The phylogenetic distribution of CSD suggests it is ancestral: it is found in the Ichneumonoidea, Apoidea, Vespoidea, Formicoidea, and Tenthredinoidea (Cook & Crozier, 1995). There are, however, insufficient data to establish whether multiple-locus or single-locus CSD is ancestral (Asplen et al., 2009). It is tempting to suggest that the mating systems of these groups should be confined to outbreeding. However, the prevalence of single-locus CSD as well as the type of mating systems found within each of these superfamilies is largely unknown. In at least some species of Ichneumonoidea (e.g., *Cotesia glomerata*; Kitano, 1976; Tagawa & Kitano, 1981; Gu & Dorn, 2003; and *C. vestalis*; de Boer et al., 2015) there is evidence suggesting a limited amount of local mating. Whether these species represent exceptions or whether local mate competition (LMC, Sect. 1.11.2) exists without inbreeding is unknown.

However, many parasitoid species inbreed with no apparent production of diploid males. In these species, sex appears to be determined by a mechanism other than single-locus CSD (Cook, 1993a). Single-locus CSD has been ruled out for nine species of Chalcidoidea, Cynipoidea, and Chrysidoidea (see references in Cook, 1993a). It is possible that the presumed absence of single-locus CSD has allowed members of these superfamilies to evolve mating systems incorporating inbreeding and LMC. Again, it is important to note that differences in the mating systems between the Ichneumonoidea and the Chalcidoidea (for instance) are largely unknown.

Multiple-locus CSD may explain sex determination in some of these cases, although to date there is no strong evidence for this mechanism. Multiple-locus CSD is similar to single-locus CSD except that more than one locus is involved in determining sex. Individuals homozygous at all of the sex loci are diploid males. Individuals heterozygous even at only one of the loci develop into diploid females. Inbreeding under multiple-locus CSD leads to increased diploid male production, but at a much slower rate than single-locus CSD. Consequently, detection of multiple-locus CSD requires several generations of inbreeding. Multiple-locus CSD has been suggested for *Cotesia vestalis* and *C. rubecula* (de Boer et al., 2008, 2012; Weis et al., 2017), *Diachasmimorpha longicaudata* (Carabajal Paladino et al., 2015; see also Asplen et al., 2009) and tested for, but not detected, in *Nasonia vitripennis* (Skinner & Werren, 1980) and *Goniozus nephantidis* (Cook, 1993c).

Finally, a more recently developed model of sex determination in the Hymenoptera is based on genomic imprinting (Poirié et al., 1993, cited in Beukeboom, 1995). Under this model, one or more loci are imprinted paternally or maternally. Fertilised eggs have a paternally imprinted copy of the genome which initiates female development (for details see van der Zande & Verhulst, 2014). This model has been confirmed and the

mechanism described for *Nasonia vitripennis* (Verhulst et al., 2010; see also Dobson & Tanouye, 1998, Sect. 3.4).

Although sex is determined by allelic diversity in probably the minority of haplodiploid species, it is thought to be the ancestral sex determination mechanism in the Hymenoptera and is better understood, both in terms of theory and in empirical work, than its alternatives: nevertheless, much remains to be discovered about the underlying mechanisms and the consequences of sex determination for mating systems.

5.3.4 Comparative Studies

While some studies look for relationships between variables within species, others look for relationships across species: these are 'comparative studies' (introduced in Sect. 1.2.3). Comparative studies may make use of the results of many behavioural and/or genetic studies and thus offer a means of assessing generality or testing otherwise untestable predictions, but often they sacrifice experimental rigour. The data of interest from the literature are often collected for different reasons unrelated to comparative study purposes. As a result, the quality of data is often variable, sometimes seriously weakening the validity of the conclusions that can be drawn from comparative studies. Furthermore, information on within-species variation will be ignored when species averages are used.

The necessity of controlling for phylogeny in such studies is now well established (Garamszegi, 2014). First of all, phylogenetic controls are necessary to take into account statistical non-independence of species that are closely related. Furthermore, controlling for phylogeny enables the distinction between interspecific similarities having a real evolutionary meaning and being simply the result of common ancestry. Methods such as phylogenetically independent contrasts (PIC) and phylogenetic least squares (PGLS) consider relatedness between species when looking at relationships between variables (asking, for instance, whether parasitoid oviposition strategy is associated with host specialization; Boulton & Heimpel, 2018). Recent developments in phylogenetic comparative methods include techniques such as ancestral state reconstructions and phylogenetic path analysis, which can help when disentangling evolutionary correlations between complex sets of traits (Gonzalez-Voyer & von Hardenberg, 2014). Advanced phylogenetic comparative methods have become increasingly easy for a non-specialist to implement, using R packages such as *ape* (Paradis & Schliep, 2018) and *phytools* (Revell, 2012) and the computer program *BayesTraits* (Meade & Pagel, 2022).

Despite these advances, few studies have sought to investigate evolutionary correlations between mating systems and associated traits in parasitoids. The few phylogenetically controlled studies so far have considered evolutionary correlations between behavioural reproductive strategies (Wajnberg et al., 2003), between clutch size and sex ratio (Smart & Mayhew, 2009), and clutch size and the female mating rate (Ridley, 1993b), with the former and the latter showing a significant association. Ridley (1993b) showed that in gregarious (multiple eggs per host) parasitoids females tend to mate multiply (polyandry) while solitary (single egg per host) species are monandrous (females mate only once). Godfray (1994) proposed that the likelihood and costs of becoming sperm depleted resulted in the evolution of polyandry in the parasitoids, as gregarious species have greater sperm requirements as they lay more fertilised eggs (daughters) over their lifetime. As more, better-quality, data are accumulated on the parasitoids, phylogenetic comparative studies will offer ever greater potential for trying to disentangle complex correlations of traits that drive, and are driven by, the evolution of parasitoid mating systems.

5.4 Parasitoid Mating Systems

5.4.1 Introduction

Natural enemies are generally small and, although desirable, it may be very difficult to study a mating system in its entirety. An overall

idea of how common local or non-local mating may be obtained by fitting a predictive model for partial local mating to sex ratio data (Debout et al., 2002; Gu & Dorn, 2003; see also Read et al., 1992, 2002, for related discussions of the selfing rates of Protozoa). Mating systems can be divided into several component parts and investigation of one of these can prove useful. Investigation of more than one component is, of course, even more useful. Good examples of studies studying several component parts of mating systems and employing a variety of techniques are Myint and Walter (1990) and Fauvergue et al. (1999). Ware and Compton (1994a, b) used sticky traps to investigate dispersal of female fig wasps in the field: similar techniques can be employed to gain insight into parasitoid mating systems (e.g., Asplen et al., 2016). Combining these with molecular techniques to assay genetic structure of populations is a particularly powerful way to understand natural enemy mating systems in more detail (Antolin et al., 2003; Gu & Dorn, 2003).

5.4.2 Emergence and Mating at the Natal Site

Temporal and spatial patterns in the emergence of adult natural enemies are important aspects of mating systems. Offspring that develop in a group, whether sharing the same host (gregarious) or on separate hosts clustered together (quasi-gregarious), may have the opportunity of mating with other group members on maturity. Offspring developing singly in isolated hosts (solitary) do not.

Emergence

The sexes may emerge simultaneously or males (protandry) or females (protogyny) may emerge first within an offspring group or population. There are few reports of protogyny in parasitoid wasps (Hennessey & West, 2018), but protandry is widespread (Boulton et al., 2015). Males generally develop more rapidly than female conspecifics and are also generally smaller (Hurlbutt, 1987; Hirose et al., 1988; Ramadan et al., 1991; Godfray, 1994; Hardy & Mayhew, 1998a, Sect. 2.9.2). Small male size may reflect selection for protandry, or protandry may be an incidental consequence of maternal sex-allocation strategies that lead to males developing in smaller hosts (King, 1993; Moynihan & Shuker, 2011).

Protandry in gregarious and quasi-gregarious species is often, but not always, associated with a high degree of mating at the emergence site, as males generally remain and mate with females as these emerge (see Bourdais & Hance, 2019, for a notable exception). Protandry can be studied by simple field or laboratory observations of developing broods or 'populations' (Kitano, 1976; Tagawa & Kitano, 1981; Nadel & Luck, 1985, 1992; Hirose et al., 1988; van Dijken et al., 1989; Kajita, 1989; Myint & Walter, 1990; Ramadan et al., 1991; Pompanon et al., 1995; Ode et al., 1996; Fauvergue et al., 1999; Hardy et al., 1999, 2000; Loch & Walter, 1999, 2002; Gu & Dorn, 2003; Moynihan & Shuker, 2011; Bourdais, 2012; Kapranas et al., 2013; Asplen et al., 2016; Bourdais & Hance, 2019), and recent studies have employed video tracking software to more accurately track emergence and dispersal (Kapranas et al., 2013). However, in some parasitoids mating may take place within the confines of the host or the host's covering (e.g., puparium; within-host-mating, WHM), which may obscure some details of mating behaviour (Suzuki & Hiehata, 1985; Tepidino, 1988; Drapeau & Werren, 1999; Leonard & Boake, 2006; Trienens et al., 2021). This complication does not occur with either quasi-gregarious endoparasitoids (e.g., Nadel & Luck, 1985, 1992; Myint & Walter, 1990) or ectoparasitoids, although ectoparasitoids mating within pupal cocoons may be difficult to observe (Tagawa & Kitano, 1981; Dijkstra, 1986; Hardy et al., 1999). Protandrous male bethylids (ectoparasitoids of many Lepidoptera and Coleoptera of economic importance) often inseminate unemerged females by chewing an entrance hole in the female's cocoon (Griffiths & Godfray, 1988; Hardy & Mayhew, 1998a) and the behaviour, and the resulting holes, can be readily observed before female emergence. However, female bethylids may also mate post

eclosion, which is exclusively the case in *Goniozus triangulifer* (Legaspi et al., 1987).

Male and female emergence patterns have been studied in *Habrobracon hebetor*, a species noted above to exhibit severe inbreeding depression. In a laboratory study, Antolin and Strand (1992) found that males and females emerged and dispersed from a brood throughout the day; no difference was found in the emergence patterns of males and females. Females tended to disperse from the emergence site before males and most females left the natal area unmated, suggesting dispersal acts as a mechanism to curtail sib-mating and reduce costly inbreeding under single-locus CSD (Ode et al., 1998).

Fauvergue et al. (1999) assessed emergence patterns of *Leptopilina heterotoma* and *L. boulardi* (Hymenoptera: Eucoilidae), parasitoids of frugivorous *Drosophila* larvae, by rearing out wasps from bananas exposed for two weeks in fruit orchards and then taken to the laboratory. Across all patches (bananas) males and females emerged simultaneously over about a ten-day period. Although in each species both sexes emerged from virtually every patch during this period, individual parasitoids frequently emerged without any conspecifics of the opposite sex emerging from the same patch on the same day. These observations were supplemented by a laboratory experiment in which offspring laid within a 24-h period were reared out and individually observed until emergence using an automated image analysis system. Adults emerged over a five-day period and both species were highly protandrous. Similar studies on other parasitoids of flies have documented protandry as well as prolonged female emergence over several days (Legner, 1968; Nadel & Luck, 1985). Bourdais and Hance (2019) studied emergence patterns in the quasi-gregarious aphid parasitoid *Aphidius matricariae*. In this species, males emerged only slightly before females in general, but protandry was extreme within broods. Even when a female laid a batch of eggs within a short period, brothers and sisters rarely emerged at the same time, which likely constitutes a mechanism to reduce inbreeding risk under single-locus CSD.

Local mating

Some models developed to predict sex allocation optima assume that when the offspring of several females (foundresses) develop in spatial aggregation, mating occurs exclusively between group members (strict local mating) and randomly, with respect to genetic relatedness (e.g., Hamilton, 1967; Table 5.1). Under these conditions, sex ratios are predicted to increase, from very low proportions of males towards approximate sex-ratio equality, as the number of foundresses increases. There have been many tests of this relationship using individual parasitoid species (e.g., Werren, 1983; Waage & Lane, 1984; Strand, 1988; Burton-Chellew et al., 2008; Abdi et al., 2020; Abe et al., 2021; Liu et al., 2023) as well as extensions of the basic theory (Hardy, 1994a; West & Herre, 1998a; Shuker et al., 2005, 2006; West, 2009; Gardner & Hardy, 2020; Lehtonen et al., 2023); in general, sex ratios become less female biased as foundress number increases, as predicted (further discussed by Orzack, 1993; Godfray, 1994; West, 2009; Boulton et al., 2015). This is because when multiple females contribute offspring to a patch the reproductive value of sons increases as mate competition among brothers over females is reduced (sons can secure matings with non-sibling females and so increase their mother's evolutionary fitness more than if only sisters are available as mates).

Several comparative studies have examined cross-species relationships between foundress number and offspring sex ratio in species with strict local mating. The best-known of these have employed natural variation in foundress number in fig-pollinating wasps (Agaonidae), which are not insect natural enemies, but share much biology with parasitoid wasps (e.g., Herre, 1985, 1987; Herre et al., 1997; West & Herre, 1998b). In these species (winged) females enter a fig, lay eggs and die (their corpses can be counted to estimate foundress number) while males are wingless and unable to disperse from the natal fig (providing what has been thought to be good evidence for strict local mating. However wingless males in some non-pollinating fig wasps do disperse; Cook et al., 1999; Greeff & Ferguson,

1999; Bean & Cook, 2001; and see Greeff et al., 2003 for evidence of male dispersal in pollinating fig wasps). Their sex ratios support the predicted relationship across species (and within species, Frank, 1985a; Herre, 1985; but see Kathuria et al., 1999; Chung et al., 2019). Despite the enormous interest in parasitoid sex ratios, there have been relatively few comparative studies and the evidence for strict local mating in other species is less clear than for fig-pollinator wasps where males cannot leave the natal fig (see Martel et al., 2016, for more discussion). Griffiths and Godfray (1988) examined a predicted relationship between sex ratio and clutch size in bethylid wasps, assuming both strict local mating and that offspring groups are produced by single foundresses. We discuss this more fully below (Sect. 5.4.5).

Random local mating

The assumption of random within-group mating will be violated if mating with non-relatives is avoided and/or if non-siblings are less likely to encounter one another (due to asynchronous maturity resulting from asynchronous offspring production by mothers; Bourdais & Hance, 2019). Sex ratios more female biased than under random mating are predicted (Stubblefield & Seger, 1990; Nunney & Luck, 1988; Table 5.1, Fig. 5.3; see also Kinoshita et al., 2002). Conversely, if individuals prefer non-siblings as mates, sex ratios are predicted to be less female biased (Stubblefield & Seger, 1990; Table 5.1; see Boulton et al., 2015, for a review of the evidence for kin discrimination in parasitoids). Of course, these predictions only apply to offspring groups produced by more than one foundress.

There have been few empirical investigations of within-group mating patterns. One of these examined mating patterns in fig-pollinating wasps. Mating is difficult to observe directly as it occurs within the fig. Frank (1985b) cut figs into halves, marked one male from each half and then joined two halves from different figs together. Males tended to remain in the half-fig of their origin, suggesting sibling-biased mating within figs and a possible explanation for observations of fig wasp sex ratios more biased than predicted by models assuming random within-group mating (Frank, 1985b).

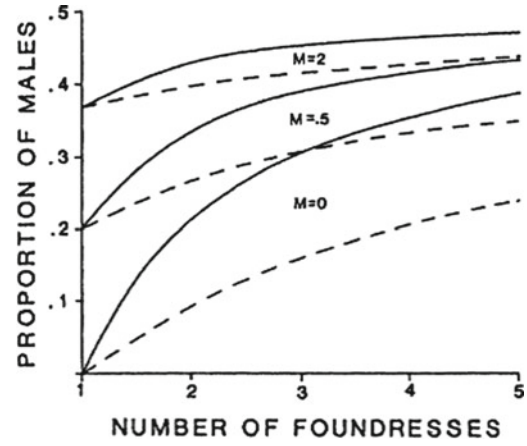

Fig. 5.3 Predicted sex ratio optima in response to foundress number, maturation asynchrony and non-local mating. The model assumes haplodiploid genetics and that, after all females in the natal patch are mated, males disperse to seek virgin females in other patches. The degree of non-local mating is represented by M, the number of patches found by a dispersing male. Solid lines show the prediction assuming offspring maturation is synchronous and dashed lines are for asynchrony. Increasing foundress numbers, increasing non-local mating and increasing synchrony each lead to less female-biased sex ratios. *Source* Nunney and Luck (1988). Reproduced by permission of Elsevier Science

Molbo and Parker (1996) and Burton-Chellew et al. (2008) used genetic techniques (allozyme polymorphism and MSATs, Sect. 5.3.3) to estimate foundress numbers in patches of hosts in natural populations of *Nasonia vitripennis*, a pteromalid wasp-parasitising fly pupae. In this species, males are protandrous and mate with females on emergence (e.g., King et al., 1969; Leonard & Boake, 2006; Trienens et al., 2021). Males also have reduced wings and are probably unable to disperse significantly from the emergence site so mating is thought to be mostly local (references in Hardy, 1994a). Molbo and Parker (1996) found variation in the timing of emergence of adult wasps, and that this was correlated with the sex ratio. In some patches of hosts,

emergence of all adults was highly synchronous and occurred in one day, while in other patches emergence was highly asynchronous, with adult wasps emerging over periods of up to 8 days (although most wasps emerged within four days). This variation across patches is most likely a result of different foundresses ovipositing on patches at different times.

As predicted by theory (Fig. 5.3), sex ratios in patches with longer, asynchronous emergence were more female biased than in patches with short, synchronous emergence (Molbo & Parker, 1996; Fig. 5.4). This is because if early emerging males do not disperse, they will remain on the patch as competitors for later emerging males. A female that oviposits on a patch later than other foundresses will maximise her fitness by producing a higher proportion of daughters (since her sons will face greater competition from non-dispersing males). The importance of asynchronous oviposition has since been integrated into LMC theory (asymmetric LMC; Shuker et al., 2005, 2006), and empirical studies have shown that *N. vitripennis* females take into account the age of other broods on a patch when allocating sex (Shuker et al., 2005, 2006).

5.4.3 Dispersal from the Natal Site

While properties such as protandry and mating at the natal site (local mating) can be demonstrated within the confines of a glass vial, such observations deny individuals the opportunity to disperse prior to mating, and thus may overestimate the importance of local mating. Modelling studies predict that dispersal and non-local mating can strongly influence parasitoid sex ratios (Table 5.1, Fig. 5.3), but empirical investigations are relatively scarce (reviewed by Hardy, 1994a). Field observations and indirect monitoring are possible in some species (e.g., Tagawa & Kitano, 1981; Kajita, 1989; Loch & Walter, 1999; Asplen et al., 2016;). In the laboratory, the propensity for, and timing of, dispersal can be investigated by simply allowing emerging individuals to leave the vial, or similar methodology (Dijkstra, 1986; Myint & Walter, 1990; Forsse et al., 1992; Fauvergue et al., 1999; Hardy et al., 1999, 2000; Loch & Walter, 2002; Gu & Dorn, 2003; Moynihan & Shuker, 2011; Bourdais, 2012; Kapranas et al., 2013; Bourdais & Hance, 2019; Böttinger & Stökl, 2020). Dijkstra (1986), studying *Colpoclypeus florus* (Hymenoptera: Eulophidae), a gregarious

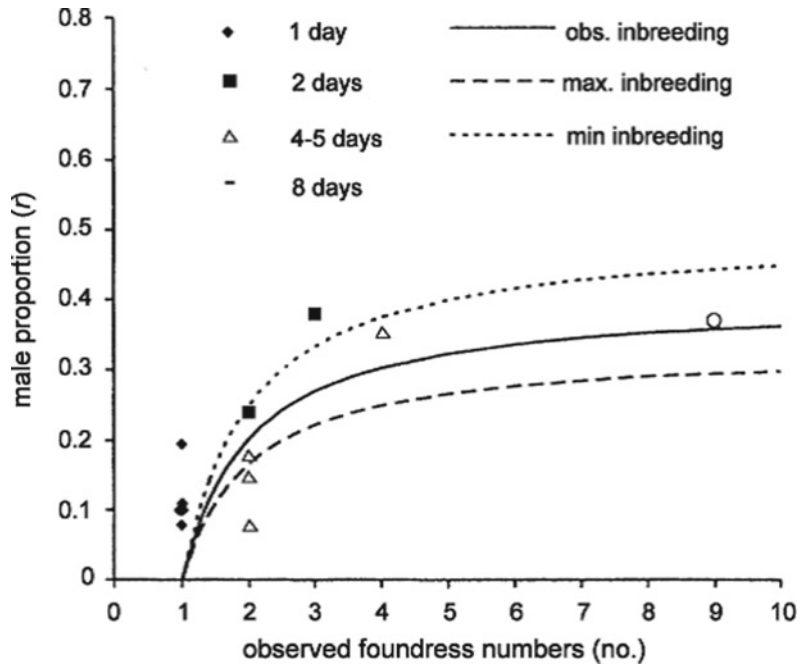

Fig. 5.4 Sex ratios in patches of *Nasonia vitripennis* in the field in relation to foundress number and synchrony of emergence within each patch (1–8 days). Curves show the predicted optimal sex ratio with inbreeding coefficients of 0 and 1 (dashed lines) and observed inbreeding (F = 0.31) (solid line). *Source* Molbo and Parker (1996). Reproduced by permission of The Royal Society of London

parasitoid of tortricid leaf-roller larvae, Hardy et al. (1999) studying *Goniozus nephantidis* (Hymenoptera: Bethylidae), a gregarious larval parasitoid of lepidopteran coconut pests, Hardy et al. (2000) studying *Goniozus legneri*, a parasitoid of lepidopteran almond pests, and Bourdais and Hance (2019) studying the aphid parasitoid *Aphidius matricariae* all found that virtually all males and females dispersed from the natal site (Figs. 5.5 and 5.6). Females collected post dispersal can be provided with hosts (e.g., Hardy et al., 1999, 2000) or the contents of their spermathecae can be examined following dissection (Nadel & Luck, 1985, 1992; Dijkstra, 1986; Ode et al., 1998) to assess whether or not they have mated. Populations of parasitoids may have several levels of organisation ranging from individual broods to sub-populations to the entire population. Populations of *Habrobracon hebetor* are frequently subdivided and approximately half of the females disperse from sub-populations before they have mated (Ode et al., 1998).

5.4.4 Post-Dispersal Mating: Natal Site Immigrants and Emigrants

Although the above methods (Sect. 5.4.3) can demonstrate dispersal and the prevalence of local mating, they do not assess post-dispersal mating; i.e., it is unknown whether dispersing males obtain matings elsewhere or, similarly, whether females mate or re-mate after dispersal. They also do not allow the possibility of mating by immigrants at the natal patch. Several studies have been carried out in which a fuller range of behaviours is possible, ranging from laboratory cages, through much larger enclosed spaces, to semi-natural and natural field populations. Comparative methods can also be used to explore relationships between the expected degree of local mating and the sex ratio (Sect. 5.4.6).

Laboratory cages

Vos et al. (1993) observed groups of *Leptomastix dactylopii*, a parasitoid wasp that develops quasi-gregariously in isolated patches of hosts (citrus mealybug) emerging in a laboratory cage. Almost all parasitoids left the natal patch before mating and at least some subsequent (off-patch) mating was observed. Both males and females were attracted to small traps containing members of the opposite sex. *Cotesia glomerata* also show partial off-patch mating (50% of females disperse without mating). Gu and Dorn (2003) filmed *C. glomerata* emergence in laboratory cages and scored emergence and mating in the natal patch using this method. Later work has shown (again using laboratory observation cages) that male *C. glomerata* do not disperse, but female dispersal is motivated by interactions with kin (Ruf et al., 2011). This study also found that dispersing females were more likely to mate than philopatric females, and they tended to disperse to patches with mating opportunities rather than control patches where no males were present.

Fauvergue et al. (1999) observed emergence and dispersal of the congeners *Leptopilina heterotoma* and *L. boulardi* from patches within laboratory cages. They found that the sex ratios produced by these species aligned with their emergence patterns: the sex ratio was not strongly female biased as predicted by LMC, likely because males emerged before females and dispersed soon after emergence, suggesting non-local mating. More recently Böttinger and Stökl (2020; see also Quicray et al., 2023) have extended this study, showing that across four species of *Leptopilina*, dispersal is a strong predictor of the volatility of sex pheromones. Species that disperse from the natal patch to mate must find mates at other locations (Sect. 5.4.5) and so greater investment in signalling and reception of cues is expected. Species that disperse from the natal patch soon after emergence produce more volatile sex pheromones which are detectable over a longer range compared to congeners which mate on the natal patch.

Similarly, pilot studies of *Goniozus nephantidis* in table-top sized cages indicate that males developing in all-male broods disperse and immigrate into broods of females. Non-sibling mating readily occurs, both in and around the females' natal area (S. Barnard and I.C.W. Hardy, unpublished).

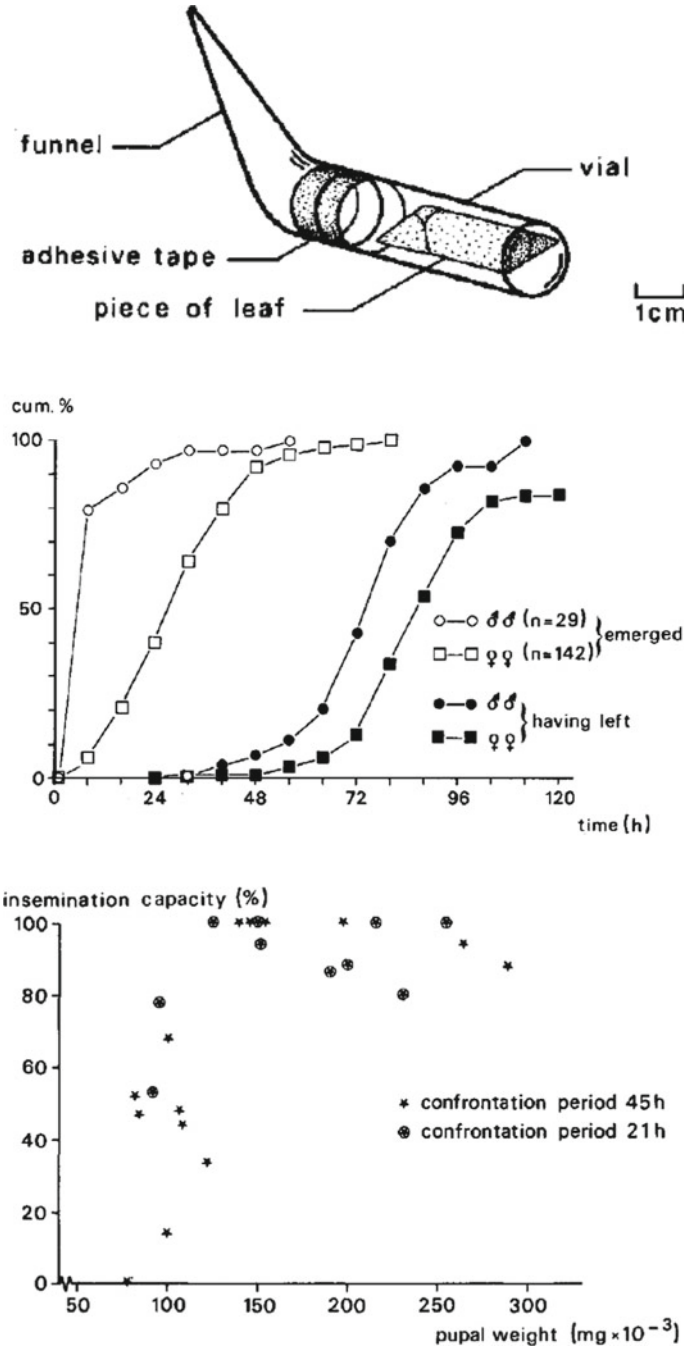

Fig. 5.5 Eclosion, local mating, and dispersal from mixed-sex broods in *Colpoclypeus florus*, a gregarious eulophid wasp in which broods develop within the web of the lepidopteran host (tortricid leaf-roller larvae). *Upper panel*: Laboratory apparatus which allows dispersal from the natal site but makes return unlikely. *Central panel*: Timing of eclosion and dispersal. Males eclose shortly before females and copulation takes place within the host's web. Both sexes disperse around two days after eclosion. *Lower panel*: Insemination capacity of males. Male body weight (estimated by pupal weight) is positively correlated with the capacity to inseminate females (males were in contact with 15 females for a total of either 21 or 45 h, although, to simulate natural emergence patterns, not all 15 females were presented simultaneously). Recently mated females do not re-mate, but it is not known whether they remain unreceptive. Although the mean sex ratio is female biased, these investigations suggest that the mating system is unlikely to conform absolutely to 'strict local mating'. *Source* Dijkstra (1986). Reproduced by permission of Brill Uitgeverij

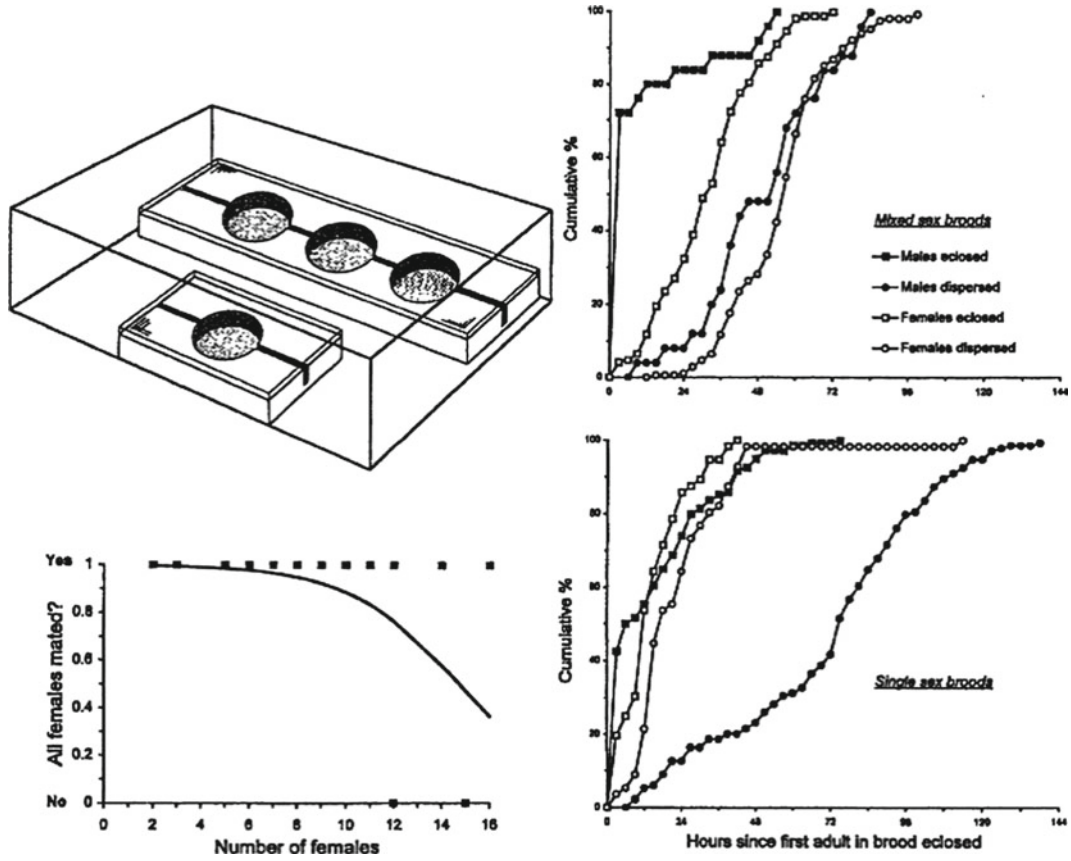

Fig. 5.6 Eclosion, local mating and dispersal in *Goniozus nephantidis*, a gregarious bethylid wasp in which broods of siblings develop within narrow feeding galleries constructed by the lepidopteran host (oecophorid larvae which feed on coconut leaves). Broods may contain both sexes or, due to male mortality, and subsequent female virginity, only one sex of offspring. Individual broods were allowed to mature in the central of three chambers in a 150 mm-long opaque plastic block decked with Plexiglass (upper left panel). A 1 mm-wide slot connected chambers and provided access to a larger transparent box from which dispersed individuals (those outside the block) were collected. A block with one chamber was also present to provide structure for dispersed adults. Observations were made every 3 h. Dispersed females from mixed-sex broods were allowed to produce offspring to assess their mating status. In mixed-sex broods, almost all females mated before dispersal (lower left panel). Males were generally protandrous, males dispersed from both mixed-sex and all-male broods, but males in all-male broods disperse more slowly (right hand panels). Virgin females dispersed from all-female broods (lower right panel). Although the mean sex ratio is female biased, these investigations suggest that the mating system of *G. nephantidis* is unlikely to conform absolutely to 'strict local mating', although field observations and/or genetic studies are required. Lower left panel: the probability that all females are inseminated in relation to the number of females eclosing from 13 broods containing only one male. The data in the lower left panel are binary (1 = all females inseminated, 0 = some females uninseminated). and the fitted curve is the probability of complete insemination, estimated using logistic regression. Although the two broods with incomplete insemination contained relatively large numbers of females the regression falls just short of significance, and in each of these only one female was uninseminated. Thus, a single male was sufficient to inseminate virtually all females, even when brood sizes were large. Similar results were obtained from *Goniozus legneri* using the same protocol (Hardy et al., 2000) and a recent study found that *G. legneri* females produced less biased offspring sex ratios when mated with males from a non-local strain (Du et al., 2021). *Source* Hardy et al. (1999). Reproduced by permission of Blackwell Wissenschafts-Verlag

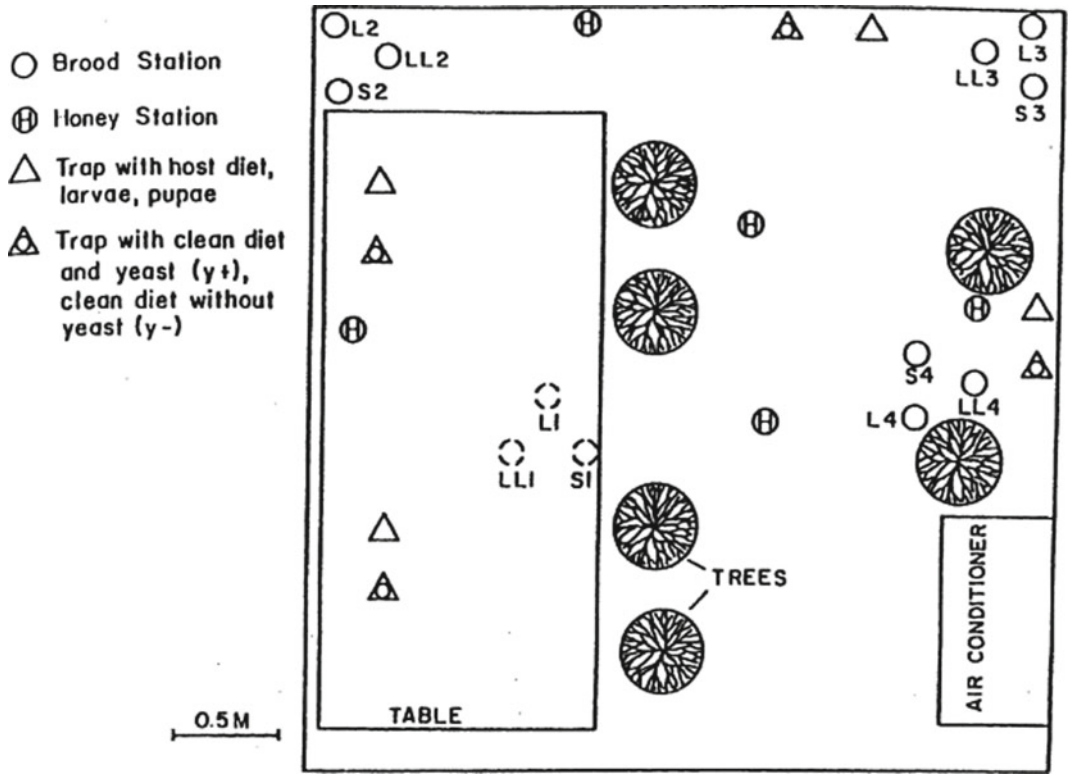

Fig. 5.7 Plan of the experimental greenhouse used to observe mating and dispersal of *P. vindemmiae*. Four groups of three single-foundress broods were arranged on the floor on slightly raised 'brood stations' (white plastic discs). Each group contained a small brood (S) of 5 hosts, a large brood (L) of 12 hosts and a large late brood (LL) of 12 hosts parasitised 2 days later than S and L. Wasps could only move between broods by flying (both sexes can fly). Wasps were not marked. Brood stations were checked every 2 hduring a 12-h period for 6–7 days (wasps were found to be quiescent outside this period). Several oviposition site 'traps' were used to catch dispersed females, which were dissected to determine whether their spermathecae contained sperm. Honey was provided but wasps were not attracted to this. *Source* Nadel and Luck (1992)

Greenhouse populations

Using greenhouse populations, Nadel and Luck (1992) investigated dispersal, immigration and mating on the natal patch and elsewhere in *Pachycrepoideus vindemmiae* (a quasi-gregarious pteromalid which parasitises *Drosophila* pupae) (Fig. 5.7). Males were protandrous and often waited for females to emerge and mate (female *P. vindemmiae* mate only once, Nadel & Luck, 1985). Males also emigrated from broods and arrived at non-natal broods, before and after female dispersal in both cases. Most females had the opportunity of pre-dispersal mating with same-patch or immigrant males, although some virgin females were collected at oviposition sites. Males also migrated to oviposition sites, where some virgin females mated. Overall, there were 26–37% extra-brood matings. Although these observations demonstrate a complex mating system, it is difficult to assign relative importance to the observed mating system components with confidence, because of unnatural elements of the experiment (further discussed by Nadel & Luck, 1992; Hardy, 1994a).

Semi-natural and natural populations

Several studies of parasitoid mating systems have been carried out in 'agricultural' environments. It might be argued that such unnatural settings could influence aspects of the mating system under investigation, because: (1) population densities are likely to be unnaturally high; (2) parasitoids are relatively poorly co-evolved with their hosts; (3) host distributions within an agricultural setting may be distorted relative to 'natural' environments. However, some parasitoids have inhabited agricultural settings for thousands of years. For instance, *Habrobracon hebetor* has been associated with pests of stored grain for at least 3,500 years (Chaddick & Leek, 1972).

Myint and Walter (1990) examined aspects of the mating system of *Spalangia cameroni* (Hymenoptera: Pteromalidae), a quasi-gregarious parasitoid of housefly pupae, in 'field' investigations carried out in a poultry shed. How closely this represents natural conditions, and thus the quantitative importance of the results, remains unclear. Males generally emerged before females developing in the same batch of hosts, and most wasps left the natal site without mating (mating was observed when the emergence of males and females happened to coincide). Olfactometer (odour choice) experiments in the laboratory (Sect. 1.6.2) showed that males were attracted to odours emanating from the host habitat. Males were also trapped in the field at patches of hosts suitable for oviposition by females (in both the presence and the absence of females) and at batches of parasitized hosts from which females were close to emerging. Sib-mating and local mating are probably uncommon in natural populations.

Loch and Walter (1999) observed the eclosion and mating behaviour of *Trissolcus basalis* (Scelionidae), a quasi-gregarious parasitoid of homopteran eggs, emerging from host egg batches placed in the field. Males are protandrous and engage in aggressive contests for egg masses. Nearly 20% of females were not mated by the dominant male on emergence, 25% of females mated multiply, sometimes with multiple males, and both virgin and mated females moved slowly from the emergence site to its surroundings before dispersing. Males also dispersed from the natal site, both during and after the period of female emergence (Loch & Walter, 2002). Both local and non-local mating probably occur in nature, but the extent of outbreeding remains unknown.

Kitano (1976) and Tagawa and Kitano (1981) observed the eclosion and mating behaviour of *Cotesia* (*Apanteles*) *glomerata*, a gregarious parasitoid of lepidopteran larvae. Observations were carried out in an agricultural system (cabbage fields). The sex ratios of most broods were female biased, although there were some all-male broods. Males tended to remain at the natal site after emergence from both brood types. In mixed-sex broods, males usually emerged first and began to search for females to court. Immigrant males arrived at both mixed-sex and all-male broods. Recently emerged females usually remained inactive near the natal site, mated within a few minutes and then dispersed. Local mating occurred but many females (likely more than 40%) mated with immigrant males, some dispersed before mating and it is unknown whether these mated elsewhere, although other field studies (references in Ikawa et al., 1993) have estimated that almost all females mate.

Van Dijken et al. (1989) observed emergence and mating of *Apoanagyrus lopezi*, a quasi-gregarious parasitoid of the cassava mealybug, in fields of cassava. Hosts occur in isolated batches. Males and females emerged from each batch over protracted periods and in random sequence. Experiments using cages of *A. lopezi* placed out in the field showed that males do not attract females or males, males are attracted to virgin females and less attracted to mated females. Males were also attracted to unparasitised hosts. *A. lopezi* probably has a panmictic mating system. Similarly, Powell and King (1984) placed cages containing adult *Microplitis croceipes* (Braconidae), a parasitoid of moth larvae infesting cotton, in cotton crops. Cages containing mated males and mated females were not attractive to free-living *M. croceipes*, but cages containing virgin females attracted males. Fauvergue et al. (1999) also used individuals in

cages to study attraction between male and female *L. heterotoma* and *L. boulardi* in the field. Cages contained virgin females, mated females, males or no individuals (control). Virgin females were by far the most attractive to free-living conspecific males.

Adams and Morse (2014) took advantage of the large size of *Alabagrus texanus*, a solitary larval parasitoid of fern moths, to study their mating behaviour in the field. They were able to mark and release males, and 'tether' virgin females to fern fronds from which they naturally emerge. They showed that males readily find and court tethered females, and that similar proportions of these females (1) mated with the first male they encountered, (2) rejected the first male but mated a subsequent male, or (3) did not mate at all. They also used this technique to assess male and female mate choice, which were confirmed under laboratory conditions.

Kazmer and Luck (1991; see also Antolin, 1999) used electrophoretic techniques (Kazmer, 1990; Sects. 3.2.2 and 5.3.3) to determine allozyme variation in populations of *Trichogramma pretiosum* and *Trichogramma* sp., parasitoids of lepidopteran eggs, and to assess the degree of non-sibling mating. *Trichogramma* sp. occurred as a natural population while *T. pretiosum* was collected from an agricultural population (tomato plots). Both species were sampled by rearing wasps from host eggs collected in the field. Although both species develop gregariously in isolated hosts and have female-biased sex ratios, only moderate frequencies (55–64%) of sib-mating were estimated. About one-third of the total mating was non-local in *T.* sp. and 2–13% in *T. pretiosum*. Further evidence for male dispersal and non-local mating is that cages of virgin females placed out in the field attracted large numbers of males (cages containing mated females did not, Kazmer & Luck, 1991). Patterns of sib-mating have been inferred using similar methods in field studies of two species with single-locus CSD, *Venturia canescens* (Collet et al., 2020; random mating with respect to sib-ship) and *Cotesia vestalis* (de Boer et al., 2015; non-random mating, suggesting sib avoidance).

Eggleton (1990) observed the emergence of a female *Lytarnis maculipennis* (Ichneumonidae) (a parasitoid of wood-inhabiting insects) in the field. Well before the female bored an exit to the surface of the wood, 4–6 patrolling males gathered to await her emergence. There were aggressive interactions between the males and one, which was larger than the others, was dominant and appeared to mate with the female while the other males apparently did not.

5.4.5 Post-Dispersal Mating: Non-Natal Mating Localities

The aforementioned studies stress the importance of the natal site as a mating locality for both emergent and immigrant individuals. However, many species normally mate in other localities, such as oviposition sites, feeding sites and even arbitrary sites. It is important to obtain information on these to fully understand the mating system.

Mating at oviposition sites

Several studies have investigated the possibility that males seek mates at oviposition sites. Some of these are summarised above (van Dijken et al., 1989; Myint & Walter, 1990; Nadel & Luck, 1992; Dias et al., 2014; see also Boulton et al., 2015; Sect. 5.4.4) and indicate that mating can occur at oviposition sites. However, Hardy and Godfray (1990) caught three species of parasitoids of *Drosophila* larvae at oviposition sites in the field and while more than a hundred females of each species were caught during the season, no males of two species and only one male of the third were caught. It is unlikely that oviposition sites are mating sites in these species. Males in at least one of these species do, however, disperse from the natal site and are attracted by virgin females (Fauvergue et al., 1999).

Mating at feeding sites

Feeding during the adult stage is common among parasitoids. In many cases hosts suitable for oviposition may serve as food for adults (e.g., Jervis & Kidd, 1986; Heimpel & Rosenheim,

1995; Heimpel & Collier, 1996; Chap. 8) and thus feeding and oviposition sites are spatially coincident. However, they may not be spatially coincident and feeding sites may serve as a mating site. Many parasitoid wasp species have been observed to visit flowers, in many cases to feed from nectar. Jervis et al. (1993) observed 249 parasitoid species visiting and feeding at flowers: 18.5% of these were represented by both males and females and thus some may use flowers as mating sites, as has been observed in nectar-feeding species of *Agathis* (Braconidae) (Belokobylskij & Jervis, 1998). Males of the desert bee-fly *Lordotus miscellus* (Bombyliidae) defend rabbitbrush plants (*Chrysothamnus nauseosus*) which the females of this parasitoid visit for feeding (Toft, 1984).

Mating at arbitrary sites: Swarming behaviour ('lekking')

The males of several parasitoid species swarm or form leks on the substrates that are not resources, e.g., *Blacus* species, *Diachasmimorpha longicaudata* (Braconidae) (Donisthorpe, 1936; Syrjämäki, 1976; van Achterberg, 1977; Sivinski & Webb, 1989), *Napo townsendi* (Braconide) (Robinson et al., 2015), *Diplazon pectoratorius* (Ichneumonidae) (Rotheray, 1981), *Aphelopus melaleucus* (Dryinidae) (Jervis, 1979) and some Encyrtidae, Pteromalidae and Eulophidae (Nadel, 1987; Graham, 1993). Pajunen (1990) suggests that swarming could have evolved from territorial behaviour when territorial systems broke down due to high population densities; however, this would not apply to all cases of swarming, e.g., *A. melaleucus* (M.A. Jervis, personal communication).

Nadel (1987) investigated mixed swarms of two species of encyrtid wasp (*Bothriothorax nigripes* and *Copidosoma bakerii*) and a species of pteromalid wasp (*Pachyneuron* sp.). Males were observed either to fly or to crawl over boulders. The swarms appeared at the same sites over a period of several weeks and contained several thousands of individuals of each species. In each species the vast majority were males. Swarms formed in the early morning and broke up during the night. Nadel (1987) established that females arrived for mating purposes: she dissected newly arriving females and found their spermathecae to be empty. Courtship and copulation occurred on boulders. Females were receptive upon arrival, were mated soon after, and became unreceptive.

Graham (1993) described the swarming behaviour of eulophids (in particular *Chrysocharis gemma*) and torymids (mostly *Torymus* spp.). Swarms comprising many thousands of individuals, the overwhelming majority being female, were observed year after year upon and around the same clump of trees, and they were present for much of the reproductive season (such site fidelity is also reported by Svensson and Petersson (2000), who studied predatory dance flies (Empididae)). The hosts of the observed species were not present locally. The ovaries of *Chrysocharis* females contained no mature eggs but it was not possible to ascertain whether their spermathecae contained sperm which would indicate whether they were virgins (van den Assem, 1996). The function, if any, of these swarms remains unknown: they may not be mating swarms at all. Syrjämäki (1976) observed swarms of *Blacus ruficornis* and found these never to contain females (>8000 individuals were examined). Similarly, Quimio and Walter (2000) found that swarms of the braconid wasp *Fopius arisanus* occurring in tree canopies were comprised of sexually immature adult males, while mature males were found in loose aggregations in lower-storey vegetation.

More recently Robinson et al. (2015) described facultative lek formation in the braconid *Napo townsendi*. Males did display on leaf tops singly, but also appeared in groups (leaves provide no resources to females and so this fits the definition of a lek). Both solitary and aggregated males were successful in mating, but males in leks appear to gain some protection from predators. It may be that males calling in groups are more protected from predation (Hamilton, 1971), but that this tendency to aggregate has been co-opted for female mate choice.

Males in a genuine mating swarm may release chemical signals in concert and thus amplify the attractive effects. In mixed-species swarms, the

signals of the different species may be very similar but interspecific communication is considered unlikely. Most probably, swarms are not formed in response to active long-distance signalling by the participants, rather, certain peculiarities of the site itself attract both sexes when the insects are receptive to mating. Nadel (1987) mentions that the sites she observed were on the only low, yet abrupt, peak within a 7.5 km radius of a mountain ridge. The sites were no more profitable in terms of food or oviposition than the surrounding areas, but they offered a distinct landmark and a certain degree of shelter from strong winds. This 'hilltopping' behaviour is known in many insects (Thornhill & Alcock, 1982). Likewise, many matings in *Habrobracon hebetor*, which attacks stored product pyralid moths, occur after dispersal from the natal site. Both males and females exhibit a prolonged period of unwillingness to mate during which time individuals disperse from the natal site (Ode et al., 1995). Males aggregate on mounds on the surface of the grain and most matings appear to occur on these (Antolin & Strand, 1992). Sometimes, these aggregation sites are coincident with higher concentrations of hosts that will attract females.

As a final point regarding dispersal behaviour, we would like to stress that, while several studies have documented the dispersal of males and females from a natal site or even a population, it is equally important to determine the fate of dispersing individuals. The one study that we are aware of concerns dispersal of the mymarid egg parasitoid, *Anagrus delicatus* (Antolin & Strong, 1987). By marking individuals, these authors were able to document female-biased dispersal to small islands more than 1 km from the nearest potential source population. The number, sex, and mating status of immigrants founding a new population have all been modelled as important variables in determining whether a new population will become established or extinct (Hopper & Roush, 1993) (see Sect. 7.4.3 in relation to biological control introductions).

5.4.6 Comparative Studies of Local and Non-local Mating

Some studies have examined cross-species relationships between sex ratio and one important aspect of mating systems: the degree of local mate competition (LMC; mating at the natal site). A recent cross-species study has also addressed how the level of LMC influences the evolution of insemination capacity across the parasitoids (Sect. 5.4.7), showing that males in species that experience high LMC can generally inseminate more females than they are expected to encounter (perhaps due to low levels of off-patch mating, asynchronous emergence and sperm competition; Martel et al., 2016). Nyabuga et al. (2012) investigated the role of resource exploitation and ant attendance on local mating in three species of aphid parasitoid (*Lysiphlebus hirticornis*, *Pausia pini*, and *Aphidius ervi*). They found that local mating scaled with the proportion of aphids parasitized within a patch (and that this was related to ant attendance, which may be used as a cue for patch quality). All three species have been reported to produce a female-biased sex ratio, but quantitative comparisons according to the predicted levels of LMC are yet to be made. Other studies have considered the sex ratio and mating system across congeneric species which experience different levels of LMC due to ecological factors, such as differences in within-host mating or male dispersal ability (Suzuki & Hiehata, 1985; Kazmer & Luck, 1991; King & Skinner, 1991; B. Stille & E. D. Parker, unpublished). A study by Stille and Parker (on gall wasps; despite being herbivores, they share much biology with parasitoids) used both allozyme techniques and morphological differences to test the relationship between the sex ratio and the degree of LMC. Suzuki and Hiehata (1985) assessed mating systems by direct observations, Kazmer and Luck (1991) used genetic (allozyme) techniques (Sects. 5.3.3 and 5.4.4), and King and Skinner (1991) used interspecific differences in wing morphology of *Nasonia*

vitripennis and *N. girault*i to infer male dispersal ability. Differences in estimated levels of non-local mating and sex ratio were in qualitative agreement with theoretical predictions in three studies, but King and Skinner (1991) found differences in the opposite direction (the species in which males are flightless had less biased sex ratios than the species with winged males). The interpretation of all four studies suffers from complications (discussed in the original papers, and by Hardy, 1994a) and from the low statistical power obtained from comparing only two species (i.e., the high likelihood of interspecific correlations between sex ratio and mating structure occurring by chance). However, an advantage of the aforementioned studies is that they were specifically designed to be comparative studies of mating systems. Some larger-scale comparative studies (see below) have been forced to use data from many sources, which often were not gathered with respect to research on mating systems. Several recent studies have compared more than two species, thus improving statistical power, and have also employed phylogeny-based analyses (Sect. 5.3.4).

Drapeau (1999) and Drapeau and Werren (1999) compared mating behaviour and sex ratio in 17 strains of three species of *Nasonia*: *vitripennis*, *longicornis* and *girault*i. They documented that within-host mating occurs in all three species, with the mean percentages of females mating within the host being 1.0%, 9.1% and 64.4% respectively. Higher levels of within-host mating are very likely to be correlated with higher levels of local mate competition, and thus more female-biased sex ratios. Drapeau and Werren (1999) examined the sex ratios produced by single foundresses and found that *N. girault*i had more biased sex ratios than *N. longicornis* and those of *N. vitripennis* were even less biased. More direct examination of the mating system thus accounted for the apparently anomalous result (King & Skinner, 1991, see above) that the species whose males had the longest wings (suggestive of a greater degree of male dispersal capability and thus less intense local mate competition) had the more female-biased sex ratio. Further comparative studies of these three species (Leonard & Boake, 2006, 2008) plus the relatively recently described *Nasonia oneida* (Trienens et al., 2021) have examined patterns of within-host mating, WHM, male behaviour and re-mating by females. For instance, inter-male aggression in defence of the emergence site of females correlates with their opportunities to mate with emerging females: in *N. girault*i, females almost always mated within the host and males were not aggressive and dispersed from the emergence site; in *N. vitripennis* females almost always emerged unmated and males were highly aggressive and did not disperse; and *N. longicornis* was intermediate for both characteristics (Leonard & Boake, 2006). Exit holes from the host are usually chewed by the males in these species and whether these are chewed before or after mating determines the natural frequency of WHM. The artificial creation of exit holes reduced WHM in *N. girault*i (Trienens et al., 2021).

Hardy and Mayhew (1998a) extended Griffiths and Godfray's (1988) comparative study of the relationship between sex ratio and clutch size in bethylids to include more species and further consideration of bethylid mating systems. Based on the general biology of the Bethylidae, which indicates that most offspring develop in single-foundress broods and that mating is predominantly local, Griffiths and Godfray (1988) made a working assumption of strict local mating. Using cross-species comparisons, they tested the prediction that average sex ratio will equal the reciprocal of average clutch size (i.e., one male per clutch, based on the assumption that one male adequately mates all of his sisters). Sex ratio and clutch size data were obtained from the published literature. Griffiths and Godfray (1988) found qualitative support for the prediction but treated species data as statistically independent (with the justification that sex ratio is likely to be an evolutionarily labile character in bethylids; indeed there is much evidence for within-species adaptive variation). Although phylogenetic constraints upon sex allocation decisions are unlikely, confounding correlations with an unknown variable may have generated spurious significance (Ridley, 1989).

While local mating almost certainly predominates in the Bethylidae, males and unmated females in some species disperse and some non-local mating seems likely (Hardy & Mayhew, 1998a). Hardy and Mayhew (1998a) reasoned that as bethylid offspring groups are usually produced by single foundresses (due to aggressive host and post-oviposition brood defence; although see Abdi et al., 2020; Gardner & Hardy, 2020), the influence of non-local mating on sex ratio should be marked (Nunney & Luck, 1988; Fig. 5.3). and potentially detectable in addition to the relationship with clutch size (Fig. 5.8). In the absence of direct evidence, sexual dimorphism in body size was used as an estimator of male dispersal ability relative to females (which must disperse from the natal site) as this potentially correlates with non-local mating. Dimorphism estimates were obtained by measuring specimens from field collections, cultures and, mainly, entomological museums. As predicted, sex ratios across species were less female biased in species with larger males (large relative to expected male size across bethylids; males were usually smaller or similar in size to conspecific females). This result was due mostly to differences between two bethylid subfamilies: Epyrinae have larger males and less female-biased sex ratios, and Bethylinae have smaller males and more female-biased sex ratios. While the species values are consistent with the adaptive hypothesis, differences between the two subfamilies not considered may also account for the cross-species trend. To carry out a phylogeny-based analysis (Sect. 5.3.4), an estimate of phylogeny was obtained from the taxonomic literature (Fig. 5.8). This was poorly resolved within genera and consequently few independent contrasts were obtained (Fig. 5.8). Analysis of contrasts found no significant relationship between sex ratio and dimorphism. Furthermore, there was no significant relationship with clutch size in the most restrictive analysis, although it was confirmed in a phylogenetic analysis using data from further species. These results are probably attributable to the low statistical power obtained from a poorly resolved phylogeny. The relationships between sexual dimorphism, non-local mating, and sex ratio are tantalisingly, but inconclusively, suggested. These comparative studies have the potential to be revisited and improved by the use of newer, and likely better, estimates of the phylogenetic relationships between the species included and addition of data from more species (i.e., Carr et al., 2010). Further, some species that were included in both studies, and that had some of the largest brood sizes and most female-biased sex ratios, would likely best be excluded: species in the genus *Sclerodermus* commonly exhibit multi-foundress broods and their sex ratios may be influenced by factors other than, or additional to, local mate competition (Tang et al., 2014; Kapranas et al., 2016; Iritani et al., 2021; Guo et al., 2022, 2023; Malabusini et al., 2022; Lehtonen et al., 2023).

A similar approach was taken in a field study testing the influences of foundress number and non-local mating on the sex ratios of non-pollinating fig wasps (West & Herre, 1998a; see also Hardy & Mayhew, 1998b; Fellowes et al., 1999; Mayhew & Pen, 2002). Many non-pollinating species are parasitoids of pollinating fig wasps. Unlike pollinator wasps (Sect. 5.4.2), female non-pollinating fig wasps lay eggs into a number of different figs from the outside. Consequently the number of foundresses contributing offspring to a particular fig is much less apparent than in pollinator species. West and Herre (1998a) predicted foundress number using a model considering three possible distributions (even, random and aggregated) of foundresses foraging for oviposition opportunities among figs. For all three scenarios, the proportion of figs in which a wasp species occurs is positively related to the average number of females laying eggs into each fig. They then used this proportion as an indirect estimate of foundress number in a test of the relationship between foundress number and sex ratio across 17 Panamanian species. Field samples found a positive relationship as predicted and as already found among pollinator species (Sect. 5.4.2). However, males in 10 species are wingless (as in pollinators) while in the other 7 males have wings. Both types mate within the fig, but winged males disperse concurrently with the females, presumably to forage

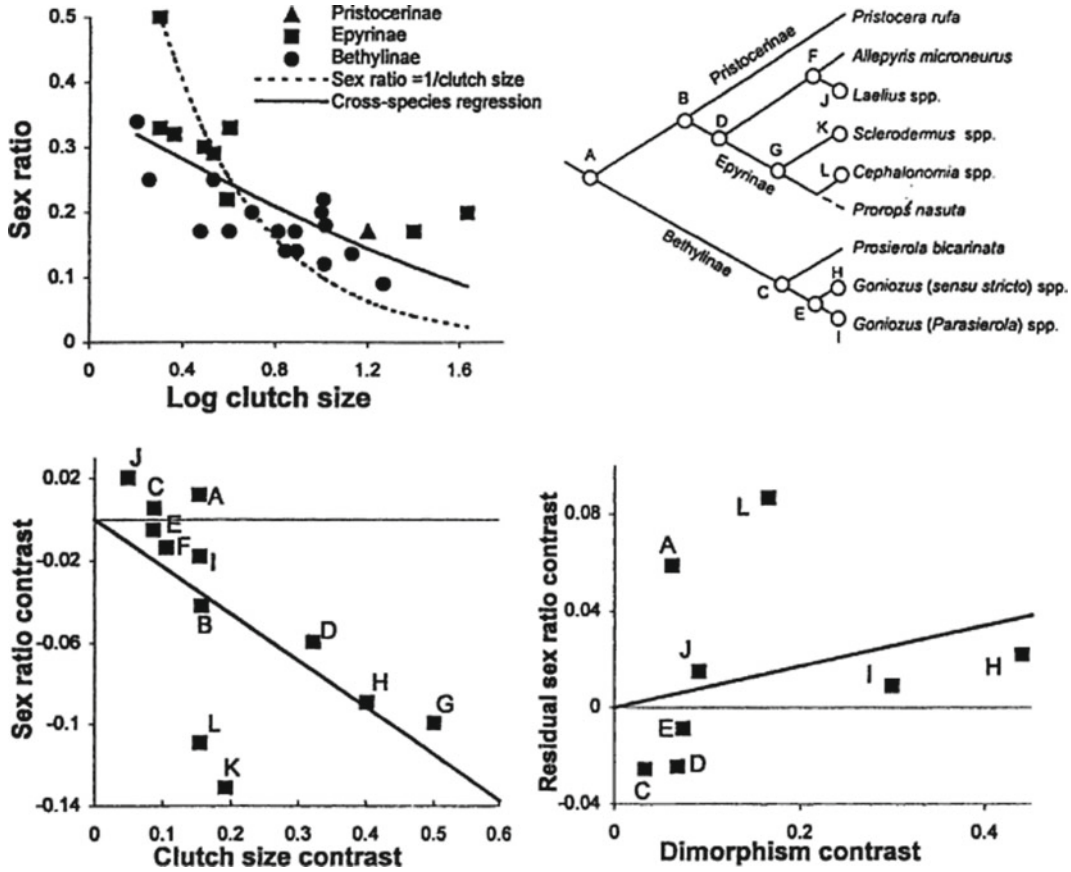

Fig. 5.8 A comparative investigation of mating structure and sex ratio in bethylid wasps. Treating species as statistically independent samples (i.e., ignoring phylogeny constraints) found a negative relationship between sex ratio (proportion of offspring that were males) and clutch size (upper left panel) and that degree of sexual dimorphism (used as a proxy for male dispersal ability and thus an indirect estimate of mating structure) was also related to sex ratio, suggesting that non-local mating influences sex ratio optima. Since cross-species analyses may be flawed, the analysis was repeated using the phylogeny-based method of independent contrasts (Sect. 5.4.4) generated using species data in conjunction with an estimate of bethylid phylogeny (upper right panel, the letters at the nodes refer to the contrasts plotted in the lower panels). Because the phylogeny estimate is poorly resolved, the number of independent contrasts for analysis is much reduced compared to the species values data. Phylogeny-based analysis confirmed the relationship between sex ratio and clutch size (lower left panel). The effect of sexual dimorphism was no longer significant, although sex ratio contrasts were generally higher when males were relatively larger (lower right panel). *Source* Hardy and Mayhew (1998a), in which full analytical details are given. Reproduced by permission of Springer Verlag

for non-local mating opportunities (however, see Greeff & Ferguson, 1999; Bean & Cook, 2001, for evidence of dispersal of wingless male non-pollinating fig wasps, and Greeff et al., 2003, for pollinators). For a given estimated foundress number, the sex ratios of species with winged males were less female biased than those of wingless-male species, a result consistent with predictions of theory considering non-local mating (Nunney & Luck, 1988; Fig. 5.3).

West and Herre's (1998a) cross-species analyses were supplemented with phylogeny-based methods (Sect. 5.3.4). The phylogeny of the species is partially known from molecular studies (Herre et al., 1996) but incomplete resolution resulted in only 9 contrasts. The effect of

foundress number survived the loss in statistical power but no significant effect of male morphology was shown. As with the Bethylidae (Hardy & Mayhew, 1998a), this difference makes interpreting the data difficult because the cross-species result could be due to other differences between taxa that happen also to differ in male morphology, and reduced power from unresolved phylogenies emerges as a common problem.

In a closely related study, Fellowes et al. (1999) examined sex ratios of 44 species of Old World non-pollinating fig wasps, which included eight species in which all males are winged, 32 in which all males are wingless, and four species in which males are dimorphic. The mean sex ratio of wingless-male species was lower than the mean sex ratio of male dimorphic species, which was in turn lower than that of the winged-male species (Fig. 5.9). The result that sex ratio is positively related to 'wingedness' was also found

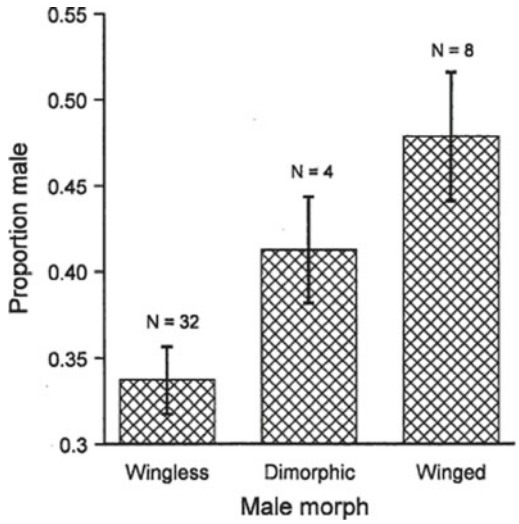

Fig. 5.9 Mean sex ratios (± standard error) of 44 species of Old World non-pollinating fig wasps. In 32 species all males are wingless and unable to disperse from the natal fig, so mating is strictly local. In 8 species all males are winged and non-local mating is likely to be the norm. In 4 species both wingless and winged males are found, and a mixture of local and non-local mating is expected. In accordance with theoretical predictions, sex ratios are positively related to the expected degree of non-local mating. *Source* Fellowes et al. (1999). Reproduced by permission of Springer Verlag

using phylogenetically based analysis, despite an incompletely resolved estimate of phylogeny (Fellowes et al., 1999, see also Hardy & Mayhew, 1999).

The aforementioned studies were aimed primarily at understanding relationships between sex ratio and mating system, and employed sexual dimorphism as an indirect estimator of non-local mating. Related comparative investigations have focused more directly on sexual dimorphism itself and found relationships between wing dimorphism and the probable mating opportunities of winged and wingless males in fig wasps (Hamilton, 1979; Cook et al., 1997). Female fig wasps have wings. In pollinator species all males are wingless. All males of a given non-pollinator species may have wings or be wingless (as in West & Herre's, 1998a, study, see above). Some non-pollinator species have both types of males (Hamilton, 1979; Cook et al., 1997). With few known exceptions, winged males mate outside the fig and wingless males mate inside (Cook et al., 1997; West & Herre, 1998a, provide evidence for winged males mating within figs; Greeff & Ferguson, 1999; Greeff et al., 2003, provide evidence of dispersal of wingless males). Since wings make movement inside the fig awkward and their manufacture requires resources, winglessness is thought to represent an adaptation to within-fig mating. In species with small broods (here 'brood size' describes the number of conspecifics developing in a fig, but these may be the progeny of a number of mothers), most females develop without any conspecific males sharing their natal fig and thus most mating must be non-local. Among these, species with only winged males predominate. Conversely, winged males are rare in species with larger broods: males are usually present in the natal fig and thus within-fig mating is more common. In male dimorphic (parasitic) species, winged males are more common in species with larger proportions of females developing in broods without males (Cook et al., 1997).

Hamilton (1979) developed a model to explore more formally the observed relationship between male dimorphism and mating

opportunities within each species. Assuming that each member of a brood is the progeny of a different mother and that females mate with either wingless males (before dispersal) or winged males (after dispersal), the model predicts that if the male morphs (alternative tactics, Sect. 5.3.1) have equal fitness the proportion of winged males should be equal to the proportion of females which develop in figs without wingless males. As the assumption that each offspring in a fig is laid by a different mother is likely to be incorrect in the vast majority of cases, Greeff (1995, 1998) extended the model to incorporate multiple-egg oviposition. When a female lays several eggs into a fig, her wingless sons may compete with each other for mating opportunities (Hamilton, 1967), while winged sons do not. Thus, individual wingless sons generate less fitness for mothers than do winged sons, and Greeff (1995) consequently predicted that the proportion of winged males may exceed the proportion of females developing in broods without wingless males. In eight out of nine male dimorphic species examined by Cook et al. (1997) the proportion of winged males equalled or exceeded the proportion of females developing in figs without wingless males. It is thus likely that the ratios of winged and wingless male morphs in these species have indeed been selected by the relative mating opportunities of the different morphs.

The link between male wing polymorphism and mating systems has also been considered by Yashiro et al. (2012) across three recently reported species of *Trichogramma*. The solitary species, *T. kurosae*, produces a greater proportion of winged males than the two quasi-gregarious species (*T. tajimaense* and *T. semblidis*), which do not need to disperse to secure matings. These data are yet to be considered quantitatively, in the light of Hamilton's (1979) model, nor has the sex ratio (which is expected to be more female biased in the quasi-gregarious species with fewer winged males, e.g., Nunney & Luck, 1988) been formally assayed.

Finally, Eggleton (1991) used a morphometric analysis to predict the type of mating system found in the Rhyssini, a monophyletic tribe of ichneumonid wasps. He measured the shape of the male gaster of specimens from nine species of Rhyssini that have been shown to exhibit one of three mating systems: males aggregate around emergence sites of females, males guard female emergence sites, and males gather around a recently emerged female. Males mate with females before female emergence in the first two systems. Eggleton (1991) found that the male gaster showed an allometric change in shape with an increase in body size in species exhibiting a mating system where males competed for mates before females emerged; no correlation was found with the other two mating systems. Eggleton (1991) argued that morphometric analyses can be used to predict the mating systems of species where no direct observations are currently available. How applicable this technique is to other taxa remains to be seen.

5.4.7 Female Receptivity and Male Ability

Females of haplodiploid species do not have to mate to be able to reproduce, but virgin, as well as sperm-depleted females, are constrained to produce only male offspring. Selection may also favour females that mate multiply. Multiple mating will almost certainly be favoured in males, but males may be constrained by limited mating ability. In this section we examine these issues in relation to mating systems.

Constrained oviposition

Whether constrained oviposition is advantageous, neutral, or disadvantageous depends on both the mating system and the sex ratios produced by other females (Godfray, 1990, 1994; Hardy & Godfray, 1990; Godfray & Hardy, 1993; Heimpel, 1994; Godfray & Cook, 1997; Hardy & Maalouf, 2017; Lehtonen & Schwanz, 2018). In panmictic populations with unbiased sex ratios, male and female offspring are of equal value and fitness costs to constrained reproduction are thus expected to be negligible. Virgin reproduction might even be advantageous if there are energetic, time or mortality costs associated

with mating, or with the production of female offspring (Nishimura, 1997; Rautiala et al., 2018; see also Chevrier & Bressac, 2002). However, if constrained oviposition becomes common, the population sex ratio may become male biased and male offspring will have less fitness than daughters due to frequency-dependent selection (Fisher, 1930; Bull & Charnov, 1988; Gardner, 2023). This may select for increased mating by eclosing females and/or for mated females to produce a higher proportion of daughters among their progeny (Godfray, 1990). Further, females might monitor the time between eclosion and mating and use this as an estimate of the level of virgin reproduction in the population. If mating status does not affect oviposition rate, an assumption that has been met in at least some species (e.g., Ode et al., 1997; Boulton & Shuker, 2015), virgin reproduction becomes more common as the time between eclosion and mating increases and females, once mated, are predicted to produce more female-biased progeny sex ratios.

The most thorough examination of the role of constrained oviposition in parasitoid mating systems has been a series of laboratory and field studies of the stored products pest natural enemy, *Habrobracon hebetor* (Guertin et al., 1996; Ode et al., 1997). As noted earlier, individual *H. hebetor* females produce female-biased sex ratios, yet several mechanisms reduce the likelihood of inbreeding (Ode et al., 1995; Antolin et al., 2003; Garba et al., 2019). Field studies following two populations over three years were conducted to examine whether the proportion of constrained (virgin or sperm depleted) females was consistently high enough to select for female-biased sex allocation and if mated (unconstrained) females were able to track fluctuations in the proportion of constrained females in the population (Ode et al., 1997). Males and females were counted weekly at eight locations at each field site. Females were brought into the laboratory where they were given several hosts each day until they died. Examination of the progeny allowed determination of whether females had stores of sperm at the time of collection as well as what sex allocation decisions they were making. At any given time, 20–30% of the females collected from the field were found to be constrained (either sperm depleted or virgin). Guertin et al. (1996) suggested that newly emerged females may experience a trade-off between remaining on the surface of the grain and finding a mate or going beneath the grain surface in search of hosts. This may explain the consistently high levels of virginity found in field populations. For each site and sample date, the observed proportion of constrained females was used to calculate a predicted sex ratio value (see Godfray, 1990) that was compared to the observed sex ratio decisions made by mated females from the collection date/site. While the proportion of constrained females appeared to explain much of the observed female bias in individual sex allocation decisions, mated females did not appear to be able to track fluctuations in the proportion of constrained females over the course of the season (Ode et al., 1997). Likewise, mated females held overnight with either virgin or mated females produced similar sex ratios, suggesting that females were not able to track levels of constrained oviposition and instead may have been responding to a long-term average.

The relationship between the time between eclosion and mating and the sex ratio has been tested in a laboratory study using *Aphelinus asychis* (Hymenoptera: Aphelinidae), a solitary parasitoid of aphids, in which mating is probably panmictic and the age of females at mating varies (Fauvergue et al., 1995, 1998). Virgin females were presented with mating opportunities at 1, 8 or 15 days after emergence, or were denied mating opportunities altogether (laboratory longevity is about 38 days). All females were provided with 100 hosts each day until death, and their progeny reared out and sexed. In accordance with the assumption that mating status does not affect oviposition rate, age at mating did not affect fecundity. As predicted, post-mating progeny sex ratio became more female biased with increasing age at mating (Fauvergue et al., 1998; Fig. 5.10). Similar patterns are reported in other parasitoids (Hoelscher & Vinson, 1971; Rotary & Gerling, 1973) but the interpretation of these results is hampered by uncontrolled variables (discussed in Fauvergue et al., 1998).

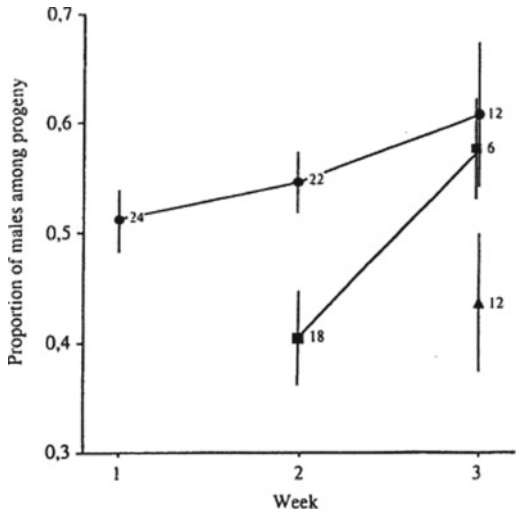

Fig. 5.10 Progeny sex ratio (mean proportion males ± standard error, with sample sizes) as a function of time from emergence (age in weeks) of female *Aphelinus asychis* mated at one day old (filled circles), at 8 days (squares) and at 15 days (triangles). Generally, mated females produce lower sex ratios when mated at an older age. *Source* Fauvergue et al. (1998). Reproduced by permission of Birkhäuser Verlag AG

When populations experience high levels of local mate competition (LMC; Hamilton, 1967, Sects. 1.11.2 and 5.4.2), optimal progeny sex ratios are female biased, virginity is thus expected to be extremely disadvantageous to individual females and mated females are predicted to make no, or very minor, adjustments to their progeny sex ratios in response to virgin reproduction in the population (Godfray, 1990). Godfray and Hardy (1993; see also Hardy & Godfray, 1990) explored the prevalence of virginity in relation to mating system across 24 parasitoid wasp species, using gregarious development as an estimator of local mating (gregarious species are generally expected to experience LMC while solitary species generally will not). Contrary to expectation, virginity was more prevalent among gregarious species (these comparisons were not phylogeny based, Sect. 5.3.4; and there are examples of gregarious species that do not experience LMC). However, it was implicitly assumed that developmental mortality was absent. Under LMC, developmental mortality can lead to considerable levels of virginity in gregarious species, because if no males survive to maturity the resulting brood of females will be virgins. Extra 'insurance' males can be produced, but at the cost of daughters (Green et al., 1982; Heimpel, 1994; Nagelkerke & Hardy, 1994; West et al., 1997; Hardy et al., 1998; see also Khidr et al., 2013; Martel et al., 2016). Under optimal sex allocation, high levels of virginity are expected when clutch sizes are small and developmental mortality is common (Heimpel, 1994; West et al., 1997). These predictions are generally supported by tests within gregarious parasitoids with a range of clutch sizes and developmental mortality rates (Morgan & Cook, 1994; Hardy & Cook, 1995; Hardy et al., 1999), but comparative analyses of the correlation between mortality and virginity using the mean values from these species gives equivocal results (dependent upon whether cross-species or phylogenetically based methods are used, Sect. 5.3.4; Hardy et al., 1998). Virginity levels across 53 species of fig wasps are negatively correlated with brood size, using both cross-species and phylogeny-based comparative methods (West et al., 1997).

Female sperm depletion and polyandry

Sperm-depleted females have mated but have used up sperm stored in their spermathecae. Like virgin females, they are unable to fertilise their eggs and are thus constrained to produce only male offspring. Depending on the mating system, this may or may not be disadvantageous (Godfray, 1990; Sect. 5.4.5; see also Cook & Crozier, 1995, for the influence of sex determination mechanisms on selection for polyandry). When constrained oviposition is disadvantageous, sperm-depleted females are expected to re-mate (Leatemia et al., 1995), and females with stored sperm may also mate again to reduce the probability of future sperm depletion (Chevrier & Bressac, 2002). However, although re-mating has been shown to replenish and increase sperm stores in many parasitoid species (i.e., *Spalangia endius*, King, 2018; *Cephalonomia hyalinipennis*, Pérez-Lachaud, 2010; *Dinarmus basalis*, Chevrier & Bressac, 2002), facultative re-mating does not appear to be widespread (Boulton et al.,

2015; Abe, 2019). In a study of field-collected females, nearly 75% of mated *Habrobracon hebetor* females became sperm depleted at some point before the end of their reproductive lifespan, as evidenced by the production of only sons (Ode et al., 1997). Fewer than 5% of these females were willing to re-mate after sperm depletion. Therefore, once they become sperm depleted, females are likely to remain constrained to produce only sons for the rest of their lives. Similarly in *Lariophagus distinguendus*, females that mated with sperm-depleted males experienced reduced daughter production but did not re-mate (Steiner et al., 2008).

A phylogeny-based comparative study, using data from 97 species of parasitoid wasps, found a significant association between polyandry (mating with multiple males) and gregarious development (Ridley, 1993b). Ridley (1993b) suggested that females of gregarious species are polyandrous to increase within-brood genetic variation among their progeny which, he argued, would decrease resource competition between siblings feeding on the same host (e.g., by resource partitioning). However, genetic diversity is also expected to promote competition as brood members have divergent evolutionary interests (Hardy, 1994b). An alternative explanation for Ridley's (1993b) result is that females are polyandrous because the extra sperm obtained reduce the risk of subsequent sperm depletion and that sperm depletion is a greater disadvantage for gregarious species than solitary species since gregarious species often inbreed while solitary mainly outbreed (Godfray, 1994; Hardy, 1994b; Boulton et al., 2015). A key test to distinguish between these explanations would be to establish whether females that mate multiple times subsequently store more sperm than do monandrous females. Possible sperm depletion appears to select for polyandry in at least one parasitoid species (*Dinarmus basalis*, Chevrier & Bressac, 2002; see Fjerdingstad & Boomsma, 1998, for an ant example). Furthermore, due to inbreeding and local mate competition, sex ratio optima of gregarious species will often be female biased, and thus require more sperm (a greater proportion of eggs must be fertilised) than solitary species with similar fecundity. Males of gregarious species may mate many times in rapid succession and themselves become sperm depleted (Sect. 5.4.5), a further reason for gregarious polyandry if Godfray's explanation applies. Most parasitoid biologists would probably favour Godfray's (1990) explanation over Ridley's (1993b), since hosts are often entirely consumed by gregarious broods and significant resource partitioning seems unlikely. Further, polyandrous females would be expected to seek genetically diverse mates if Ridley's (1993b) explanation were correct, and this may influence the mating systems of gregarious species by encouraging outbreeding (further discussed by Hardy, 1994b).

One way of distinguishing between the alternative explanations would be to re-categorise the solitary species as 'truly solitary' (developing singly in hosts, hosts isolated) or 'quasi-gregarious' (developing singly in hosts, hosts in clumps). Interaction of developing siblings cannot occur in quasi-gregarious species (classified as solitary by Ridley, 1993b) but in their mating systems they are likely to be similar to gregarious species: Godfray's (1990) hypothesis predicts polyandry while Ridley's (1993b) hypothesis predicts monandry (Hardy, 1994b). Since only two of the 68 'solitary' species in Ridley's (1993b) study are reported as polyandrous, if many of these 68 species were quasi-gregarious, the support would be for Ridley's (1993b) hypothesis. Ridley's (1993b) study was hampered by the generally poor knowledge of parasitoid mating behaviour and a low-resolution phylogeny. Although better supported molecular phylogenies are now available for many parasitoid families (i.e., Heraty et al., 2013), extensions of his work are likely to suffer from similar issues when compiling trait data. This again illustrates that, despite the vast knowledge of parasitoid biology in general, there is a great need for basic data on mating systems and mating behaviour.

Godfray's (1990) hypothesis could only explain the incidence of polyandry if female parasitoids do indeed become sperm depleted. There is evidence that females can be very

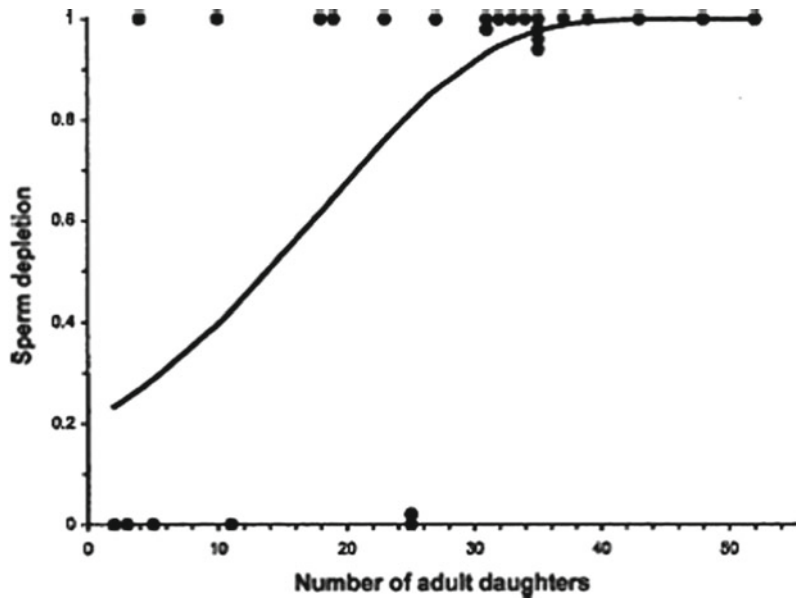

Fig. 5.11 Sperm depletion of female *Cephalonomia hyalinipennis* in relation to the number of adult daughters produced (hosts were provided in batches of 10 hosts [4 prepupae plus 6 pupae] every 3–4 days). Data are binary (0 = female died with no evidence of sperm depletion, 1 = female was sperm depleted before death [only males emerged from a batch of hosts, and no further daughters were produced]). Where multiple data points overlap, some are displaced from their binary positions to show sample sizes. The curve illustrates the probability of becoming sperm depleted, estimated using logistic regression. *Source* Pérez-Lachaud and Hardy (1999). Reproduced by permission of Elsevier Science

economical with their stored sperm, with as few as one spermatozoid released per fertilised egg in *Habrobracon hebetor* (*H. juglandis*) (Braconidae), a parasitoid of pyralid moths and *Dahlbominus fuscipennis* (Eulophidae), a parasitoid of sawflies (Speicher, 1936; Wilkes, 1965). However, sperm depletion of females is reported from many laboratory studies (e.g., Gordh et al., 1983; Hardy & Cook, 1995; Luft, 1996; Fauvergue et al., 1998; Pérez-Lachaud & Hardy, 1999; Chevrier & Bressac, 2002; King & Bressac, 2010; Pérez-Lachaud, 2010). For example, Pérez-Lachaud and Hardy (1999) presented female *Cephalonomia hyalinipennis* (Bethylidae), a parasitoid of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae), with batches of hosts at regular intervals until the female died: 77% of females ran out of sperm before the end of their reproductive lives (females then produced broods containing only adult males, and no further daughters). The probability of sperm depletion was positively correlated with the number of daughters produced (Fig. 5.11). An estimate of the minimum number of spermatozoids initially stored can be obtained by assuming that only one spermatozoid is used per fertilisation and dividing the number of daughters produced prior to sperm depletion by the probability of egg-to-adult survival (Pérez-Lachaud & Hardy, 1999).

While laboratory evidence is useful, it must be borne in mind that under laboratory conditions females may be presented with an unnaturally large number of reproductive opportunities, sperm depletion may thus be overestimated, and estimates may also be affected by the re-mating opportunities presented in the laboratory. Laboratory studies will generally be more useful for assessing the numbers of spermatozoids that can be stored than for estimating the natural occurrence of sperm-depleted females. The one example of estimations of sperm depletion in the field comes from *Habrobracon hebetor* (Ode et al., 1997, see above for discussion).

Laboratory studies can also shed light on how processes other than sperm depletion can contribute to the costs and benefits of polyandry in the parasitoids. For instance, direct or material benefits appear to provide fecundity and longevity benefits to parasitoid females in some species (*Trichogramma evanescens*, Jacob & Boivin, 2005; *Nasonia vitripennis*, Boulton & Shuker, 2015). Laboratory studies can be designed in a way that allow investigators to assess and/or model the ecological salience of these processes, as well as their importance in an applied context. For instance, multiple mating in some cases appears to temporarily disrupt sperm use by female parasitoids (Flanders, 1956; *Anaphes nitens*, Santolamazza-Carbone & Pestaña, 2010; *Nasonia vitripennis*, Boulton et al., 2018a, b), resulting in an overproduction of males. Under laboratory culture conditions, where females have more opportunity to re-mate, this can be detrimental to mass rearing for biological control (Fuester et al., 2003); understanding why and how this occurs can help practitioners to design mass rearing conditions which circumvent this issue (i.e., by permitting female dispersal). By varying the context in which mating occurs, investigators can also extrapolate about the ecological importance of processes such as this. For instance, Boulton et al. (2019) found that in *Nasonia vitripennis* overproduction of sons immediately after mating was reduced under multi-foundress (low LMC) compared to single-foundress (high LMC) conditions. We may then expect that the costs of re-mating will vary according to the mating system (i.e., inbreeding and high LMC v*ersus* outbreeding and low LMC), resulting in different levels of female receptivity to re-mating across populations.

Male sperm depletion and polygyny

While selection may favour female virginity, monandry or polyandry, males will virtually always be selected to mate with many females (polygyny). A literature survey of parasitoid mating behaviour by Gordh and DeBach (1978) found that in all examined species (n = 48) males were polygynous (only 5/34 species of females were polyandrous). There have been several studies showing that body size has a positive influence on the number of females a male can successfully mate (e.g., Jones, 1982; King, 1987; van den Assem et al., 1989; Heinz, 1991; Godfray, 1994; Ode & Strand, 1995; Lacoume et al., 2006). Patterns of spermatogenesis in male parasitoids are variable across species: in many species spermatogenesis ceases before eclosion ('pro-spermatogenic' species; e.g., Gerling & Legner, 1968; Martel et al., 2008a; Martel et al., 2016), while in others it may continue during adult life (e.g., Wilkes, 1963; Laing & Caltagirone, 1969; Gerling & Rotary, 1974; Gordh & DeBach, 1976; Nadel & Luck, 1985; He & Wang, 2008; Martel et al., 2016) and, unusually, there may be a premating period during which adult males do not have sperm ready to ejaculate (Quimio & Walter, 2000). In social Hymenoptera, the testes normally degenerate upon male migration, the lifetime supply of sperm being stored in the seminal vesicles, but a few species have wingless males that continue spermatogenesis throughout life, and do not disperse and compete aggressively with other wingless males for within-nest mating opportunities (Heinze et al., 1998). Male parasitoids that mate many times in rapid succession may run out of sperm, either temporarily or permanently, and females receive no sperm or a reduced quantity (King and Schlinger & Hall, 1960; Wilkes, 1965; Laing & Caltagirone, 1969; Gordh & DeBach, 1976; Nadel & Luck, 1985; van den Assem, 1986; Dijkstra, 1986; Ramadan et al., 1991; Ode et al., 1996; Bressac, 2010; Kant et al., 2012; King, 2018).

Nadel and Luck (1985) assessed the insemination capacity of male *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae), a quasi-gregarious pupal parasitoid of *Drosophila* and other flies. One-day-old males were presented with a succession of ten 18-h-old virgin females (each female singly). Females were replaced with virgins immediately after mating. It took about one hour for a male to mate with all ten females. Mated females were either offered hosts and the sexes of their progeny recorded or dissected and the contents of their spermathecae examined. The amount of sperm transferred on mating decreased

as the number of females mated increased. In a similar experiment, males were presented with a rapid succession of mating opportunities, allowed to rest for 24 h and then presented with further virgin females. A full complement of sperm was transferred to the latter class of female, showing that males replenish their sperm supplies (spermatogenesis). The duration of the rest period was then varied: sperm replenishment following one mating was found to require about 30 min. Time between matings is thus an important influence on male insemination capacity. Nadel and Luck (1985) investigated the timing of adult emergence from batches of hosts (Sect. 5.4.2). Males usually emerged one day before females. Females tended to emerge in the mornings and the mean interval between female emergence during daytime was 125 min. In 24% of cases, females emerged less than 30 min apart, but the majority of intervals were >30 min. Thus, in nature, males are probably able to fully inseminate the majority of females that emerge in their natal patch.

Similar methods have been used to examine sperm replenishment patterns in *Habrobracon lineatellae* (Laing & Caltagirone, 1969) and *H. hebetor* (Ode et al., 1996), *Aphidius ervi* (He & Wang, 2008) and *Anisopteromalus calandrae* (Abe, 2019). Ode et al. (1996) presented males with either a high encounter rate of virgin females (until males did not mate with an additional female; about twenty females per day) or a low encounter rate (one female per day). Body sizes of the males were measured (all females were of a standard size) and all mated females were given four hosts per day until death. By rearing the progeny, the timing of sperm depletion and replenishment patterns were determined, as well as the number of daughters each male had sired. Furthermore, when mating opportunities were plentiful (10 matings per day), large males were able to deliver sperm to more females than small males, both because they were able to mate with more females and because they were able to deliver more sperm to the females that they did mate. When mating opportunities were rare (1 per day), male size had no effect on the rate of sperm depletion (Ode et al., 1996).

Rather than presenting males with virgin females individually (Nadel & Luck, 1985; Ode et al., 1996), females can be presented simultaneously, as was done by Dijkstra (1986) to assess the insemination capacity of *Colpoclypeus florus*. Individual males, of known weight, were presented with five females simultaneously for four hours, then five more virgin females were added, then after a further four hours five more females were added. Males were in contact with 5 rising to 15 females for a total of 45 h. The number, timing and duration of female presentation was chosen to reflect the emergence patterns and sex ratios that this species would naturally exhibit. In a further experiment, males were presented with females as above, but males and females were only kept together for a total of 21 h. Males in both experiments were offered another batch of five females after the main experiment to determine whether their sperm stores were replenished. Insemination was assessed by examining the contents of the females' spermathecae. The proportion of females inseminated did not depend on whether males were in contact with females for 21 or 45 h. Females that mated with a small male or a male that had mated many times were less likely to receive a full complement of sperm, suggesting that males, particularly small males, do become sperm depleted. Dijkstra (1986) concluded that, under normal circumstances, a single male is sufficient to inseminate fifteen or more females. Limited male insemination capacity is unlikely to account for the observation that an increasing number of male eggs is laid in larger clutches; the high developmental mortality in *C. florus* broods is a more likely explanation (Hardy et al., 1998).

Ramadan et al. (1991) used two methods to study male mating ability in four species of Opiinae (Braconidae), solitary parasitoids of tephritid fruit-fly larvae. In the first method, which was similar to Nadel and Luck's (1985) and Dijkstra's (1986), individual males were presented with ten virgin females for 24 h and then the females were dissected for spermathecal examination. Replicates were carried out using males of different ages and sizes. Male ability peaked at around four days and, in two species,

larger males were able to inseminate more females than smaller males. In the second method, 200 males and 200 females were placed together in cages (the studied species are solitary and may mate in swarms as well as immediately after eclosion). Every 24 h, fifteen females from each cage were dissected (fifteen males were also removed and discarded to maintain an even sex ratio). The experiment was stopped when all fifteen females in a sample were inseminated. Most females mated during the first 24 h (but some did not mate until more than 7 days old) and were observed to be polyandrous.

Some researchers have thus assessed male capacity at maximum rates of presentation of virgin females and then related this to the natural timing of female emergence (Nadel & Luck, 1985) while others first assessed emergence and then presented virgin females so as to mimic these patterns (Dijkstra, 1986). Note that these approaches are not applicable to species such as *Habrobracon hebetor* (Ode et al., 1996), where mating occurs at sites other than the site of emergence. An alternative, though closely related, approach was used by Hardy et al., (1999, 2000) and Loch and Walter (2002): eclosion and dispersal in broods of *Goniozus nephantidis* was monitored and dispersed females were provided with hosts to assess whether they were mated (Sect. 5.4.2). Brood size varied naturally and many of the broods contained only one male. Virtually all females were inseminated (Fig. 5.6). This method thus estimates the capacity of males to inseminate females as individuals mature and disperse naturally. No decisions about the timing and duration of presentation, or the number and age of individuals used, need be made by the investigators. Such decisions may influence the results if, for example, females have short periods of receptivity (Sect. 4.3.6) or if male mating ability is age dependent during the first few days of adult life (Hirose et al., 1988; Ramadan et al., 1991). However, the efficiency of this method is reduced by natural variation that may make some replicates less valuable (e.g., broods from which two males eclose), and important factors such as brood size (number of virgin females per male) are not under direct experimental control. A similar approach was taken by West et al. (1998), who examined insemination capacity in five species of fig wasp. Females that had emerged naturally in figs containing only one male were dissected. The maximum number of inseminated females in a fig was much greater than the mean number of females per fig. Males were also dissected to check whether their seminal vesicles contained sperm: none had empty vesicles. It is thus unlikely that males of these species become sperm depleted.

Male and female mate choice

Given the fitness consequences of mating with a sperm-depleted male, and the relative rarity of polyandry (Ridley, 1993b; Boulton et al., 2015), it is unsurprising that in several species females preferentially mate with males with sufficient sperm stores (He & Wang, 2008; Ruther et al., 2009; Wittman & King, 2019). These cues may be indirect and may not necessarily reflect active female choice (i.e., younger or larger males have more sperm and are more successful in securing matings), but in *Nasonia vitripennis* virgin females exhibit a preference for males which excreted greater amount of a pheromone (Ruther et al., 2009). In this species, small males and sperm-depleted males produce less pheromone and are less attractive to females (Ruther et al., 2009; Blaul & Ruther, 2012).

Low levels of polyandry across the parasitoids can also render male mate choice favourable because it reduces wasted effort courting unreceptive females. Moreover, sperm precedence is often biased towards first males when polyandry does occur in parasitoids (Holmes, 1974; Damiens & Boivin, 2006; Martel et al., 2008a), and so investing limited sperm stores in already mated females is likely to be costly. Mate choice by males for virgin females and a male aversion to mated females (or their pheromones) has been demonstrated in several parasitoid species (*Aphelinus asychis*, Fauvergue et al., 1995; *Spalangia endius*, King et al., 2005, Fischer & King, 2012, *Trichogramma euproctidis*, Martel et al., 2008b), likely as a result of these investment costs.

The parasitic Hymenoptera can contribute much to understanding of the evolution of mate choice under systems of inbreeding, both in

terms of discriminating sperm levels or kinship (i.e., Wittman et al., 2016; Wittman & King, 2019; Collet et al., 2020) as well as understanding the mechanisms underlying choice (Ruther et al., 2009; Blaul & Ruther, 2012; Xu et al., 2019). The methods used to study mate choice typically involve no-choice or choice laboratory-based trials. Selecting the appropriate methodology is important as preferences are typically reduced under 'no-choice trials' (in which only one stimulus is presented and the response, or the speed of the response, to it is recorded) compared to 'choice trials' in which two or more mate stimuli are presented (Dougherty & Shuker, 2015; Dougherty, 2020). Although choice tests are more likely to show whether individuals can discriminate between different mates and show a preference, it is important that ecological relevance is considered during study design. For instance, a no-choice scenario might be ecologically relevant for a solitary species, but not a gregarious or quasi-gregarious one. Wittman and King (2019) used a series of two-choice tests in the quasi-gregarious pteromalid *Urolepis rufipes*. In three separate experiments they presented females with males that were either (1) once mated or multiply mated, (2) one day old or 10 days old (and virgin) or (3) infected or uninfected with a pathogenic bacterium (and virgin). They used a Petri dish divided with a silicone insert as a choice arena and tested female choice, as opposed to male propensity to mate, by allowing each test male to mark one side of the arena. The males and the silicone insert were then removed and a female was introduced into the arena and the time that she spent in each side of the arena was recorded. They found that females spent more time in the side of the arena marked by once-mated males, compared to those which had mated multiply (and so were less likely to be sperm depleted). They also found that this preference was advantageous in terms of increasing daughter production. Female *U. rufipes* also preferred young males to old males, although this did not provide any fitness benefit when males were virgin; it may be that male age is an additional proxy for likely sperm depletion. Finally, females preferred males that were uninfected, but only when the choice was between males that were uninfected or infected with a low dose of bacteria (which killed only 10% of infected males). When stimulus males were infected with a high dose (which killed 50%), females switched their preference to infected males. The authors suggest that this is because at high infection levels males may increase their investment in reproduction if death is imminent, whereas low-dose males invest in immune function to overcome the infection. This may mean that pheromonal marks produced by highly infected males are more potent than those produced by uninfected males, or of males that were exposed to only low doses of bacteria. Techniques such as those used by Ruther et al. (2009), Blaul and Ruther (2011, 2012) and Xu et al. (2019) would no doubt elucidate the mechanisms underlying these patterns of choice. These studies employed dichloromethane extractions, gas chromatography–mass spectrometry (GC-MS) and gas chromatography preparative fraction collect (GC-PFC) to identify and measure volatile compounds produced by *Nasonia vitripennis* (Ruther et al., 2009) and *Cotesia glomerata* (Xu et al., 2019). Responses to these volatile cues were then measured using Y-tube or four-arm olfactometers. Different volatile stimuli are allowed to diffuse into different arms of the olfactometer, an individual is released into the apparatus and their preference recorded based on their movement towards the stimuli. An additional method employed by Xu et al. (2019) was gas chromatography electro-antennographic detection (GC-EAD), whereby the electrical output produced by an antenna is recorded in response to different olfactory stimuli. Studying the composition and volume of volatiles from signalling individuals will provide information not only on the mechanism of choice but may shed light on how choice has evolved and to what extent it represents sexual conflict (i.e., whether males mark females with anti-aphrodisiac pheromones to repel competitor males, or if female pheromonal composition changes after mating such that they are no longer attractive to males).

5.5 Predator (and Other Non-Parasitoid) Mating Systems

5.5.1 Introduction

The study of non-parasitoid insect mating systems has largely adopted the non-genetic, individual-based approach to defining and understanding an insect's mating system (Sect. 5.3.1). As outlined earlier, there are four basic types of mating system: monogamy, polygyny, polyandry and polygamy (Alcock, 1998). Monogamy is a relatively rare mating system requiring special ecological constraints. One of the best examples comes from non-predators: there are termite species in which winged sexuals establish a new colony after their nuptial flight. The colony is founded by a single male and female, both of whom dig a new nest after removing their wings. The opportunity for further nuptial flights is therefore lost, and monogamy ensues in these primary reproductives: the male and female invest huge amounts of resource into their gonads and soon become immobile, reinforcing the reliance on their subterranean partner (Wilson, 1974).

Polyandry is widespread in the insects and direct or material benefits that increase female longevity and fecundity are common (Arnqvist & Nilsson, 2000). Perhaps the most interesting examples come from social insects (e.g., Wilson, 1971) where monogamy is ancestral to taxa that have evolved obligate eusociality and currently observed polyandry is a derived state (Hughes et al., 2008a; Boomsma, 2009, 2013; Quiñones & Pen, 2017; Boomsma & Gawne, 2018; Smith et al., 2018). Newly emerged honeybee queens restrict their nuptial flights to a few days after emergence when they rendezvous with the drones from other colonies in mating swarms that aggregate high above landmarks. Females need to mate several times in order to receive enough sperm to generate a long-lived, viable colony. The combination of queen rarity at mating swarms (i.e., the OSR is highly male biased) and the need for multiple mating has led to the evolution of the peculiar copulatory mechanism in this species. Drones autotomise their genitalia once they have inserted their intromittent organ and released semen into the queen. This means that drones only get one opportunity at mating (they die soon afterwards) whilst females can mate with several males during their relatively short visits to mating swarms (Wilson, 1971). Since males only mate once, and females mate multiply, this is a polyandrous mating system.

Perhaps the best example of insect polygyny occurs in a predator, a protandrous eumenid wasp whose males defend clusters of female brood (Smith & Alcock, 1980). Males defend their cluster vigorously, and often violently, because it offers them the opportunity to mate with several females without incurring large search costs. This type of polygynous mating system (termed 'female defence polygyny') is associated with female monogamy, the ability of females to mate soon after emergence and predictable clustering of receptive females.

Polygamy is, by and large, typical in insect mating systems (Thornhill & Alcock, 1982), with males and females mating multiply throughout their lives (and thereby providing the preconditions for sperm competition, Sect. 4.5.2). There is an ever-expanding list of variants on the polygamous theme. For example, when fertilisable females are plentiful and predictable in space and time, and are defendable, we usually observe a mating system where males defend a resource that is required by females and exchange access to it for copulations. A good example of this type of mating system comes from the predatory yellow dung-fly (*Scathophaga stercoraria*), where males defend spatially restricted oviposition sites (fresh dung) which females require to lay eggs. Males fight for areas of the dung pat that maximise their opportunity to encounter sexually receptive, gravid females as they arrive to oviposit (Fig. 5.12). This polygamous mating system is termed resource defence polygamy and is relatively common (see below). Demonstrating resource defence polygamy first requires careful observations of the mating system, which should include measures of male size and mating success, as well as

Fig. 5.12 A pair of yellow dung-flies (*Scathophaga stercoraria*) copulate on a fresh dung pat. Males which are able to displace rivals from the areas around the fresh dung pat that maximise the probability of encountering a receptive, gravid female achieve relatively high reproductive success. By mounting, and hence guarding, females when they arrive, and copulating directly before these guarded females oviposit onto the dung, these males ensure they fertilise most of the eggs the female lays. The dung-fly mating system can be broadly described as 'resource defence polygamy'. *Photograph* M. Siva-Jothy

measures of those aspects of the putative resource that the observer feels are related to the mating success of the resource-holding male. In the majority of cases, defended resources are usually a spatially and/or temporally restricted food and/or oviposition resource, so measures of size and age are usually relevant. Once correlations have been established between resource variables and mating success it is a relatively straightforward matter to manipulate the key resource variable and predict mating outcomes. This general approach has been used to identify quite obscure resource variables that play a central role in determining mating outcomes (e.g., Gibbons & Pain, 1992; Siva-Jothy et al., 1995).

The non-genetic, individual-based approach adopted by students of non-parasitic insect mating systems considers three major selective forces that underpin mating system evolution. First, and perhaps ultimately, ecological factors impose constraints upon the availability of receptive females. Second, the degree to which males compete for access to females as a consequence of female availability and third, the extent to which female behaviour (choosiness) influences male reproductive success. The first of these forces is a sufficiently intuitive, large, and well-reviewed area that we will not consider further here (Thornhill & Alcock, 1982; Kokko et al., 2014, provide reviews). We will, however, briefly consider the evolutionary forces that result in male and female traits (and the conflict that sometimes arises between males and females) that subsequently refine an insect species' mating system.

5.5.2 Competition for Females

The most overt manifestation of Bateman's principle (Sect. 5.2) is the enormous range of male sexual traits devoted to securing matings and ensuring fertilisation. In the first instance this phenomenal array of male traits is manifest as 'attractiveness' traits such as acoustic (Bennet-Clarke, 1970), visual (Lloyd, 1966) and/or chemical signals (Breed et al., 1980). However, when ecological factors dictate, males supplement, or forego, signalling by actively searching out females (Smith & Alcock, 1980). In more extreme instances, males are also selected to wait in the vicinity of resources that are themselves attractive to females. In general these resources tend to be feeding sites (Severinghaus et al., 1981), oviposition sites (Siva-Jothy et al., 1995), emergence sites (e.g., Gilbert, 1976) or conspicuous aspects of the habitat (landmarks) (Downes, 1970). As the OSR becomes more male biased at these sites, selection will favour the evolution of agonistic behaviour in males directed at defending the resource that attracts females and/or the females themselves (Fig. 5.12). Males that best defend the resource will be favoured since they will have a higher encounter rate with receptive females: the stage is now set for the evolution of additional morphological and behavioural traits that enhance a male's fighting ability. However, if the OSR is too male biased the pay-offs of defence may be too low, or even negative (Alcock et al., 1978). At such times, a male-biased OSR, a mating system known as 'scramble competition', generally evolves: males are selected to get to a receptive female as quickly as possible and not waste time or energy in fighting (Smith & Alcock, 1980). The ecological idiosyncrasies of particular species can also favour the evolution of alternative male mating behaviours within a mating system. Such mating systems support two (sometimes more) male tactics for securing mates (Johnson, 1982). These alternative tactics can be underpinned by different alleles; they can have a genetic basis which triggers a shift from one tactic to the other as conditions dictate; or they can be condition dependent, in which case an individual is not obliged to express both tactics but uses the one most appropriate for its condition (reviewed by Cade, 1980). Variation within a mating system and the evolutionary conditions underpinning it have been particularly attractive to game theory modelling (e.g., Dawkins, 1980; Mesterton-Gibbons, 1999; Neff & Svensson, 2013; Abe & Kamimura, 2015; Parker, 2020).

Competition between males does not stop once a mate is secured. Because polyandry is the norm in insects, most insect mating systems generate sperm competition (Sect. 4.5.2), a selective force which favours the evolution of two suites of antagonistic male traits (Parker, 1970): one set reduces the likelihood of the female re-mating (Fig. 5.2) whilst the other increases the competitiveness of a male's ejaculate (Simmons, 2001). Both of these traits operate to reduce levels of sperm competition and can have direct effects on the nature of mating systems. For example, if males are selected to reduce the chances of female re-mating to reduce the likelihood of future sperm competition, then the OSR will become more male biased, and the foundation may be laid for an evolutionary arms race with females (since females may benefit from re-mating). Much of the variation in mating systems is generated by selection on males to enhance their ability to secure matings and fertilisations, but it is clear from this last scenario that females also play a role in determining the characteristics of mating systems: there has been a noticeable shift towards understanding female roles in shaping mating systems, particularly in insects (Rowe et al., 1994; Arnqvist & Nilsson, 2000; Pizzari & Wedell, 2013; Boulton et al., 2018a, b).

5.5.3 Choosy Females

In any mating system where females gain a net benefit from being choosy about their mating partners, males will be subjected to additional selection pressures. For example, females might be choosy because they benefit from gaining access to better nutritional (Thornhill, 1980a) or ovipositional (Siva-Jothy et al., 1995) resources or, because they obtain 'good' male genes

(Andersson, 1994). These genes may be 'good' because they have utility via parasite resistance (Zuk, 1988; Siva-Jothy & Skarstein, 1998) or developmental stability (manifested as 'fluctuating asymmetry', Thornhill, 1992; Møller & Swaddle, 1997). Alternatively, 'good genes' may provide a benefit to the choosy female via the attractiveness they confer on her sons (Jones et al., 1998). The challenge for evolutionary ecologists is to disentangle the relative importance of direct (i.e., resource-based) and indirect (i.e., gene-based) benefits to females (Arnqvist & Nilsson, 2000; Slatyer et al., 2012). A classic example of the pivotal role of female 'choice' in determining the nature of a mating system comes from studies of scorpion flies (Mecoptera) (e.g., Thornhill, 1980a). Male fertilisation success is determined by the duration of copulation, which in turn is determined by the size of the prey item that a male presents to a female (Fig. 5.13). Males that present larger nuptial gifts secure longer copulations, transfer more sperm, and transfer receptivity-reducing compounds that delay female re-mating (Thornhill, 1980b). Female-based behaviour (nuptial feeding) results in selection favouring males that can secure and defend the largest nuptial gifts. Nuptial gift giving also results in selection on polyandry, if females can acquire more resources by mating with multiple males (Arnqvist & Nilsson, 2000) and this can also depend on female condition (in many species females in poor condition re-mate at higher rates to gain greater material benefits, Toft & Albo, 2015). Insect predators are particularly useful models for studies investigating direct benefits of polyandry and mate choice as the energetic benefits of nuptial gifts combined with the high costs of hunting mean that male gift

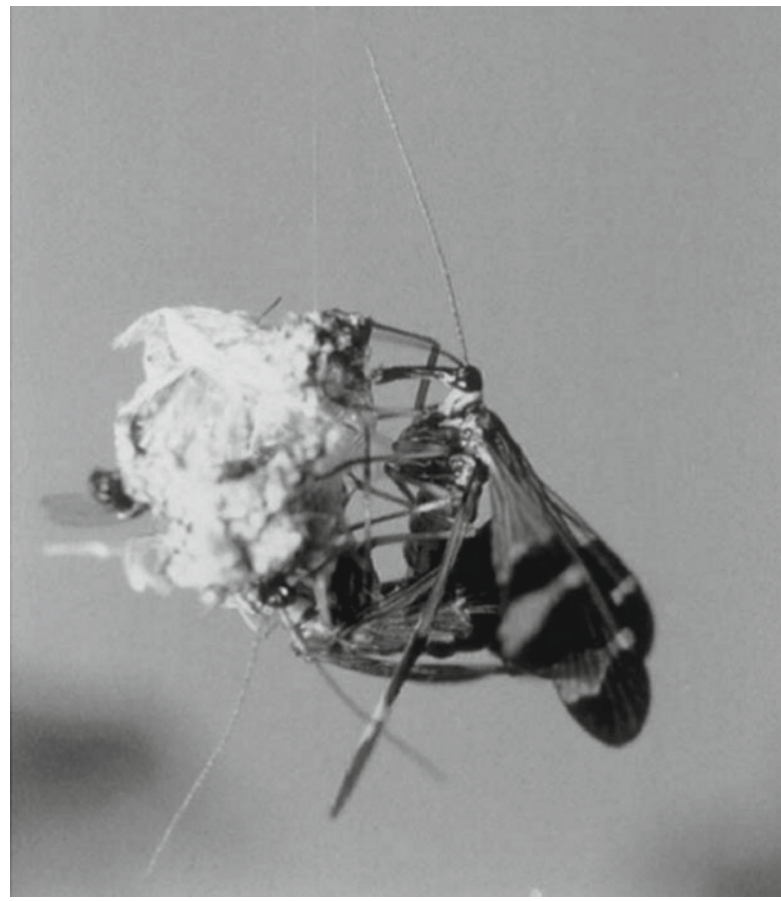

Fig. 5.13 A male panorpid scorpion fly copulates with a female whilst she feeds on the cluster of dead insects he has captured, enveloped and suspended from a leaf. Males attract females by utilising pheromones. and males with larger gifts obtain higher levels of reproductive success because females prolong copulation in order to continue feeding (Thornhill, 1980b). *Photograph* M. Siva-Jothy

giving (and the evolutionary arms races that can follow from it) is particularly likely (Ghislandi et al., 2017).

Further to this overt form of female-driven preference for male nuptial gifts, Eberhard (1996) proposed that females may choose males cryptically by biasing fertilisation events (i.e., the outcome of sperm competition, Sect. 5.2) in a particular male's favour. As well as influencing mating system parameters, cryptic female choice has been suggested as operating on the ability of a male to stimulate the female during courtship, and that this is a driving force for speciation via the rapid and divergent evolution of male genitalia (Eberhard, 1985; Hosken & Stockley, 2004).

The selective forces generated by sperm competition produce a number of male traits that do not always function in the female's interest. In many cases females can control some sperm precedence patterns (Wilson et al., 1997; Snook & Hosken, 2004; Peretti & Eberhard, 2010). By examining the variation in sperm precedence patterns in the bean weevil, *Callosobruchus maculatus*, using identical sets of paired matings between and within female sib-groups, Wilson et al. (1997) showed that some of the variance in sperm precedence (i.e., male fertilisation success) was explained by female genotype. Females therefore appear to be wresting control of fertility away from males. But male *C. maculatus* have their own agenda: males deliberately damage the female's genital tract during mating (Crudgington & Siva-Jothy, 2000). This male trait is believed to increase female reliance on her last mate's sperm by inducing reluctance to re-mate and increasing the re-mating interval. This is a particularly good example of sexual conflict since not only is the male trait easy to document and examine, but also the female shows a behavioural response (kicking), the experimental elimination of which results in increased costs for females. Examination of anatomical traits that may be involved in determining fitness consequences of mating have been dealt with in Chap. 4 (Sect. 4.5.2). A recent example, again from *C. maculatus*, shows that female genital morphology and immune function have co-evolved with male traits that inflict damage (Dougherty et al., 2017). Another spectacular example of a reproductive conflict comes from studies of the ejaculate of *Drosophila melanogaster*. Male products transferred to the female in the ejaculate incapacitate rival sperm in storage in the female, and so have clear benefit to the copulating male (Harshman & Prout, 1994; but see Holman, 2009). However, they also reduce the longevity of the female (Chapman et al., 1995; but see Holman, 2009). These substances are therefore the basis of a reproductive conflict of interest between male and female *D. melanogaster* that has led to an evolutionary arms race as intense as in any host–parasite system (Rice, 1996).

Sexual conflict is now a well-developed field which has been studied in a wide range of taxa (Chapman et al., 2003; Pizzari & Snook, 2004; Arnqvist & Rowe, 2005; Fricke et al., 2009) and has been expanded to consider genomic as well as physical conflict between males and females (Bonduriansky & Chenoweth, 2009). In the case of physical sexual conflict, the first step is always to identify a system that is likely to be under sexual conflict. To reveal whether sexual conflict is operating, studies must start by demonstrating that a reduction in fitness for one sex is co-dependent on the behaviour or physiology of the trait that benefits the opposite sex.

The mating rate is a commonly cited example of sexual conflict, as females usually have a lower optimum mating rate than males. Stutt and Siva-Jothy (2001) demonstrated this experimentally using bed bugs. They adjusted the male copulation rate to maintain maximum fertility with a minimum of copulations and showed that male-controlled copulation rates resulted in a 25% reduction in female survivorship. Similarly sexual conflict over female re-mating is ubiquitous as it reduces male fertilisation success. Male tactics such as male mate guarding are the most obvious manifestation but physiological adaptations such as 'mating plugs' are also commonplace (Stockley et al., 2020). In these cases, carefully designed studies are needed to assess the nature and strength of the conflict. For

instance, mate guarding might reflect conflict if it serves only to safeguard male fertilisation success (i.e., mate guarding *in absentia*, Sect. 5.2) but not necessarily, as it can also benefit females (i.e., protecting them from harassment or predation during oviposition or foraging; Rowe et al., 1994; Boulton et al., 2018a, b). Other techniques that have been employed more recently involve investigating species- or population-level associations between the harmful and beneficial traits (Dougherty et al., 2017) or using experimental evolution to track evolutionary arms races (Edward et al., 2010).

5.6 Conclusions

With recent advances in evolutionary thinking, and the integrated use of recently available investigational and analytical techniques, the study of natural enemy mating systems continues to provide us with: (1) insight into the evolutionary mechanisms that generate diversity in reproductive traits, (2) an understanding of the utility of those traits, and (3) an understanding of the determinants of reproductive success and optimal reproductive decisions in natural populations. Descriptions and studies of mating systems have largely been of interest to those seeking empirical support for evolutionary theory. Via effects on sex ratio and fecundity, mating systems influence natural enemy population dynamics (Sect. 7.4.3) as such, their potential for informing pest control strategies is probably under-utilised (Hardy & Ode, 2007; Ode & Hardy, 2008).

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Populations and Communities

6

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6.1 Introduction

In nature, any particular terrestrial habitat contains animal, plant, fungi, and microbe species that exist together in both time and space. Many of these species will interact with each other, for example when one species feeds on another or when two species compete for the same food or other resource. A group of species having a high degree of spatial and temporal concordance, and in which member species mutually interact to a greater or lesser extent, constitute a community (Askew & Shaw, 1986). The size and complexity of a community will depend upon how broadly that community is defined. For example, we could consider as a community the organisms which interact with each other within a particular area of woodland, the herbivore species which compete for a particular food plant or the complex of natural enemies associated with a particular prey or host species. Here, we are especially interested in communities of natural enemies, which are often surprisingly species rich (Carroll & Risch, 1990; Hoffmeister & Vidal, 1994; Settle et al., 1996; Sunderland et al., 1997; Memmott et al., 2000; Peralta et al., 2014; Wirta et al., 2014; Frost et al., 2016; Shameer et al., 2018).

The animal species of a community obtain their food directly or indirectly from plants which are the primary producers of the community. Herbivores feed directly on plants whilst predators and parasitoids are either primary carnivores, feeding on herbivores, or secondary or tertiary carnivores, feeding on other predators or parasitoids. The successive positions in this feeding hierarchy are termed trophic levels. Thus, traditionally, green plants occupy the first trophic level, herbivores the second level, carnivores which eat herbivores the third level, secondary carnivores the fourth level, and so on, although a species may occupy more than one level. For example, some insect parasitoids are facultative hyperparasitoids, thus having the potential to occupy two trophic levels. Similarly, some carabid beetles eat both insect prey and plant

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seeds, and because they are polyphagous predators, the insect prey consumed may consist of herbivores, detritivores or even other carnivores. In fact, since omnivory (feeding on more than one trophic level) is very common (Polis & Strong, 1996; Coll & Guershon, 2002) it is often more helpful to think in terms of food webs (trophic webs, or trophic networks) (Sect. 6.3.12) than trophic levels. Empirical studies show food webs to be much more complex than was previously thought (Williams & Martinez, 2000; Wirta et al., 2014). Many generalist predators are known to kill and consume other predators (Rosenheim et al., 1995) and parasitoids (Brodeur & Rosenheim, 2000). When the predators concerned also belong to the same guild, the interaction is termed intraguild predation (IGP) and the top predator benefits by obtaining a meal from the victim predator or parasitoid and by simultaneously reducing competition for the shared herbivore food resource (Polis et al., 1989). There is now a consensus that IGP amongst predators (Fagan et al., 1998; Janssen et al., 1998; Eubanks, 2001; Lang, 2003; Gagnon et al., 2011) and between predators and parasitoids (Sunderland et al., 1997; Colfer & Rosenheim, 2001; Meyhöfer, 2001; Meyhöfer & Klug, 2002; Snyder & Ives, 2003; Frago, 2016) is very common in agricultural systems, but also occurs in diverse natural systems (Denno et al., 2002; Woodward & Hildrew, 2002). Some parasitoid species can probably reduce the incidence of predation on their populations by avoiding habitat patches emanating olfactory cues associated with predators (Moran & Hurd, 1998; Taylor et al., 1998; Raymond et al., 2000; Frago & Godfray, 2014). A given predator or parasitoid taxon can vary in its degree of impact on herbivore prey populations, and thereby on plant yield (i.e., vary in its propensity to initiate a significant trophic cascade; Polis, 1999), even within a year in one type of crop (Snyder & Wise, 2001). The definition of trophic cascades has recently been updated and refined to "indirect species interactions that originate with predators and spread downward through food webs" (Ripple et al., 2016). This definition includes parasitoids as a type of predator, in line with previous efforts to unifynatural enemy roles in food webs (Raffel et al., 2008; Buck & Ripple, 2017). The incidence of trophic cascades is quite variable in natural systems, as cascades depend on a number of interacting factors, including the relative colonisation rates of herbivores, predators, and parasitoids in relation to the spatial distribution pattern of host plants (Thomas, 1989), the relative strengths of interactions in the upper trophic levels (Spiller & Schoener, 1990), and the productivity level of the system (Müller & Brodeur, 2002). These patterns are also likely to vary strongly over time (reviewed in Piovia-Scott et al., 2017). Natural enemies in the higher trophic levels can exert a positive influence not only on plant productivity, but also on plant biodiversity (Moran & Scheidler, 2002; Schmitz, 2003). Trophic cascades are very common (Morin & Lawler, 1995; Halaj & Wise, 2001; Symondson et al., 2002; Ripple et al., 2016), and occurred in 45 out of the 60 independent cases (from 41 studies) analysed by Schmitz et al. (2000). Moran and Scheidler (2002) point out that trophic cascades operate in many systems ranging from simple (e.g., arctic) to very diverse (e.g., tropical rainforests), suggesting that diversity has little effect on the strength of top-down trophic interactions. Buck and Ripple (2017) reviewed the role of parasites in trophic cascades, finding that parasitoids were the group of parasitic organisms most commonly implicated in initiating trophic cascades. Strong trophic interactions can exist in diverse systems that also encompass an abundance of weaker interactions. Available evidence suggests that intraguild predation and omnivory do not, in general, prevent generalist predators from initiating strong trophic cascades (Halaj & Wise, 2001; Costamagna et al., 2007). In addition, both direct and indirect effects of natural enemies on their prey and resulting trophic cascades can be mediated by interactions ranging along a continuum from (1) consumptive effects, due to a natural enemy consuming a host, to (2) non-consumptive or 'trait-mediated' effects, where a natural enemy does not kill hosts or prey but rather causes them

to adopt costly defensive strategies (Werner & Peacor, 2003; Preisser et al., 2005; Preisser & Bolnick, 2008; Abram et al., 2019).

In this chapter, we will focus on the elucidation of consumptive effects, for which most methodology, analytical techniques, and theory have been developed. Amongst the many methods described in this chapter there are likely to be at least a few that are appropriate for studying trophic interactions in any given agricultural or natural system. In the realm of food webs, theory abounds, and whereas empirical evidence was once scarce (Morin & Lawler, 1995), new methods are increasingly improving identification of processes influencing food web structure (Boyer et al., 2016; Frost et al., 2016; González-Chang et al., 2016). The range of methods described here will help to guide researchers in the collection of data vital for continuing to develop and test theory. Techniques useful for discovering who eats whom are outlined in Sect. 6.3. In addition, since symbiotic microbes can also be transferred from one host species to another through the links in a food web (e.g., effects of *Wolbachia* spp. propagating through herbivore–parasitoid food webs) (Werren et al., 2008; McLean et al., 2016), we describe, in Sect. 6.5, methods for the detection and identification of *Wolbachia* and other symbionts in food webs. Phytophagy by natural enemies is discussed in Chap. 8.

Species within a community exist as populations. In its broadest sense the term population can be applied to any group of individuals of the same species occupying a particular space. This space may vary greatly in size, for example from a single tree to a wide geographical area, depending upon how the population is defined. It is important to define the spatial scale over which the population is to be studied at the start of an ecological investigation, since the principal factors influencing the population dynamics of a species may vary depending upon spatial scale. For example, immigration and emigration may have a much greater influence on population persistence at small spatial scales than they do at larger ones (Dempster, 1989). The term 'population' is sometimes incorrectly used to refer to the combined numbers of a range of related species occupying a discrete area, for example the 'carabid population' of a field. Great care must be taken in the interpretation of changes in the abundance of such a 'population' since it will comprise a mixture of species with differing ecologies, and different species will be affected in different ways by the same environmental factors. The concept of a metapopulation as a collection of sub-populations of a species, each occupying a discrete habitat patch but with some level of genetic interchange, has received much attention in the study of species population dynamics (Gilpin & Hanski, 1991; van Nouhuys & Lei, 2004). This concept is discussed further in Sect. 6.4.

In order to study a natural enemy population or community it is usual to select at random a representative group, a sample, of individuals on which to make the appropriate observations or measurements so that valid generalisations concerning the population or community as a whole can be made. Often, but not always, the sampled individuals need to be removed from their natural habitat using an appropriate collection technique. Most sampling methods are destructive, involving the physical removal of organisms from the study area. It is however, sometimes possible to sample by counting organisms *in situ* (Sect. 6.2.6). It is essential that before starting a sampling programme, sampling techniques are chosen that are appropriate for both the type of ecological problem being investigated and the particular natural enemy species being investigated. Section 6.2 is devoted to a description of the most commonly used sampling techniques and their limitations, with reference to examples from the literature on natural enemies. Table 6.1 lists some applications of these techniques. Basset et al. (1997) provide a key to assist ecologists in the choice of sampling methods suitable for one specific habitat – the tree canopy. Ausden (1996) gives a table which summarises the relative applicability of various sampling techniques for a range of invertebrate groups (but not exclusively natural enemies).

In summary, this chapter is concerned with the *sampling* and *monitoring*, in time and space,

Table 6.1 Applications of different field sampling methods

Data required	Sampling technique	Comments
Absolute abundance	Pitfall traps	Do not provide data on absolute abundance
	Vacuum net	When used to sample a defined area or unit of habitat, calibration is necessary. Large, active insects may flee before sample taken. Multiple re-sampling or combination with ground search will increase accuracy
	Sweep net	Estimates of absolute abundance difficult to obtain
	Knock-down	For chemical knock-down, unit of habitat (e.g., whole plant) needs to be enclosed; calibration necessary; cannot be done when windy; ineffective for species enclosed within vegetation (e.g., galls, leaf-mines and silken retreats)
	Visual count	Labour-intensive; inects need to be conspicuous if census-walk method used; efficiency varies with insect activity and observer; insects may be absent (e.g., underground) during daytime
	Mark-release-recapture	Important to satisfy a number of assumptions; choose appropriate calculation methods
Relative abundance	Pitfall traps	Factors affecting locomotor activity, catchability and escape rate by different species, need to be taken into account
	Vacuum net	Efficiency can change with height and density of vegetation
	Sweep net	A wide range of factors cause sampling variability; significant variability between operators
	Knock-down	Except for chemical knock-down, may not sample all species with the same efficiency
	Visual count	Labour-intensive; insects need to be conspicuous if census-walk method used; efficiency varies with insect activity and observer
	Attraction	Difficult to define area of influence; insect responses may change with time
Dispersion pattern	Pitfall traps	Trap spacing important in minimising inter-trap interference; vegetation around individual traps can affect capture rates; some carabids aggregate in traps
	Vacuum net	Vegetation type can affect efficiency
	Sweep net	Disturbance can change dispersion pattern during sampling
	Knock-down	Chemical knock-down needs to be confined to defined sampling areas
	Visual count	Detectability needs to be constant over study area
	Examination of hosts for parasitism	Identification of immature stages may prove problematic

(continued)

Table 6.1 (continued)

Data required	Sampling technique	Comments
Phenology	Pitfall traps	Cannot detect immobile insects (e.g., during cold weather)
	Sweep net	Changes in vertical distribrution within the vegetation may result in non-detection
	Malaise trap	Provides useful information on flight periods
	Visual count	Changes in behaviour may affect ease of detection
	Examination of hosts for parasitism	Rearing provides information on diapause characteristics
	Attraction	Responses to visual or chemical stimuli may be restricted to certain periods or behavioural states
	Sticky traps, window traps	Only valid during active flight periods
Species composition	Pitfall traps	May not sample all species with the same efficiency; provide useful data on presence; lack of catch does not prove absence
	Vacuum net	Night samples need to be taken for nocturnal insects
	Sweep net	Only efficient for groups active in the vegetation canopy
	Knock-down	Very activeflyers may escape, but, otherwise, thecatch from chemical knock-down is not dependent on the activity of insects and is not influenced by 'trap behaviour'
	Visual count	Most efficient for very conspicuous groups
	Attraction	Different species may not respond to the same visual or chemical stimuli
	Sticky/window traps	Species without active flying stage will not be recorded
Relative abundance of species	Pitfall traps, vacuum net, sweep net, Malaise trap, knock-down, attraction, visual count	Except for chemical knock-down, may not sample all species with the same efficiency
Locomotor activity	Pitfall traps	Linear pitfall traps provide useful information on population movements, especially direction of movements; best to combine with a marking technique
	Visual count	Can provide useful information, especially if combined with a marking technique
	Attraction	Attractant properties of trap can interfere with insect behaviour
	Mark-release-recapture	Provides useful information such as minimum distance travelled
	Sticky and window traps	Provide useful information on height of flight, as well as on direction

of natural enemy populations and communities, and describes a number of techniques that can be used for measuring or estimating the abundance of natural enemies, determining the structure and composition of communities and examining the spatial distribution of natural enemies in relation to their host or prey populations. Most of the techniques discussed can be used to obtain *qualitative* data relating to the predator or the parasitoid species present in a community or the prey/host species attacked by a particular natural enemy. Some of the techniques can also be used in obtaining *quantitative* estimates of natural enemy abundance or predation and parasitism, an aspect of natural enemy biology taken further in Chap. 7.

Estimates of animal numbers may be expressed in terms of either density per unit area or unit of habitat, and the unit of habitat can be an area of ground or a unit of vegetation such as a leaf or a whole plant. Estimates of this type are termed absolute estimates of abundance and must be distinguished from relative estimates of abundance which are not related to any defined habitat unit (Southwood & Henderson, 2000). Relative estimates are expressed in terms of trapping units or catch per unit effort and are influenced by other factors (e.g., climatic conditions) besides the abundance of the insect being sampled. When considering absolute estimates of insect abundance, the term population density may be applied to numbers per unit area of habitat and the term population intensity applied to numbers per leaf or shoot or host (Southwood & Henderson, 2000).

This chapter is concerned with practical techniques and we say little about the statistical analysis and interpretation of sampling data. Information on these topics can be found either elsewhere in this book (Chap. 9) or in the following publications: Cochran (1983), McDonald et al. (1988), Perry (1989, 1998), Eberhardt and Thomas (1991), Crawley (1993, 2002), Sutherland (1996), Krebs (1999), Southwood and Henderson (2000), McGarigal et al. (2013), Young and Young (2013).

6.2 Field Sampling Techniques

Our discussion of techniques is mainly confined to the sampling of insects from terrestrial habitats and from air. Sunderland et al. (1995a) discuss sampling options for determining the density of predators in agroecosystems, and Williams and Feltmate (1992), Ausden (1996), Southwood and Henderson (2000) give fuller accounts of sampling methods for use in aquatic habitats.

6.2.1 Pitfall Trapping

A pitfall trap is a simple interception device consisting of a smooth-sided container which is sunk into the ground so that its open top lies flush with the ground surface (Fig. 6.1a). Invertebrates moving across the soil surface are caught when they fall into the container.

Pitfall traps (Fig. 6.1b) are the most commonly used method of sampling ground-dwelling predators such as carabid and staphylinid beetles, spiders and predatory mites, but have occasionally been used to sample other groups of predators, such as dolichopodid flies (Pollet & Grootaert, 1987), pompilid wasps (Field, 1992), ants (Cherix & Bourne, 1980; Samways, 1983; Melbourne, 1999; Morrison, 2002), heteropteran bugs (Rácz, 1983; Basedow, 1996) and harvestmen (Cherix & Bourne, 1980; Jennings et al., 1984; Newton & Yeargan, 2002). Abundance data for fire ants (*Solenopsis* spp.) obtained by pitfall trapping may need to be interpreted with caution in cases where these ants are attracted to build nests under the pitfall traps (Penagos et al., 2003). The containers that can be used as pitfall traps are many and varied, but round plastic pots and glass jars with a diameter of 6–10 cm are the most popular. It is important to remember, however, that both trap size and trap material are known to influence trap catches, sometimes very strongly (Luff, 1975; Adis, 1979; Scheller, 1984). That said, there are ways in which bias can be minimised and pitfall trap used to give

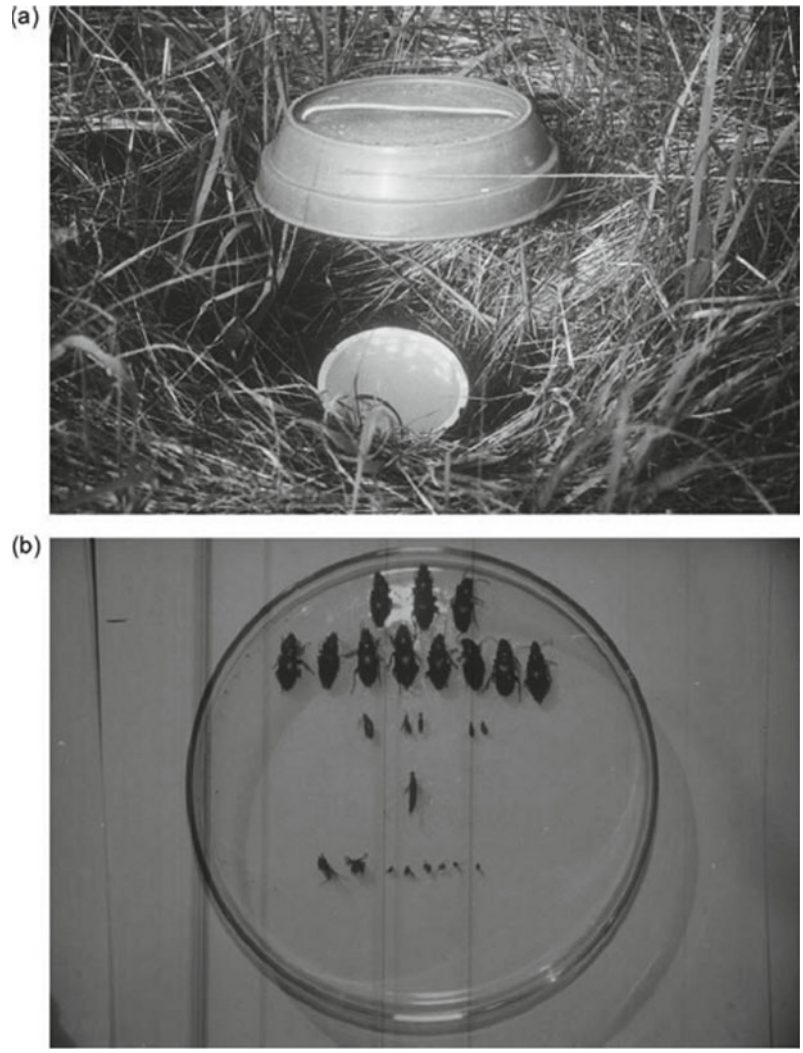

Fig. 6.1 **a** A simple pitfall trap (with rain cover) for sampling predators moving across the soil surface, e.g., carabid and staphylinid beetles and spiders; **b** carabid beetles, staphylinid beetles and spiders caught in a single pitfall trap over a 7-day period in a cereal crop in southern England

meaningful measures of abundance (e.g., Gist & Crossley, 1973; Woodcock, 2005).

A liquid preservative is often placed in the trap, both to kill and to preserve the catch, thereby reducing the risk of escape and preventing predation within the trap, particularly of small individuals by larger ones (conspecifics or heterospecifics). Amongst the preservatives that have been used are formaldehyde, alcohol, ethylene glycol, propylene glycol, acetic acid, picric acid, sodium benzoate and concentrated salt solutions, but there is evidence that some preservatives have an attractant or repellent effect on some insects and that such effects can differ between the two sexes of the same species (Luff, 1968; Skuhravý, 1970; Adis & Kramer, 1975; Adis, 1979; Scheller, 1984; Pekár, 2002). Lemieux and Lindgren (1999) showed that, although the efficiency of trapping carabid beetles did not differ significantly between concentrated brine (a super-saturated solution of sodium chloride) and antifreeze (ethylene glycol), the former was a poor preservative of predators. Addition of detergent can greatly increase the catch of small predators (such as linyphiid spiders), which may otherwise fail to break the surface film and thus be able to escape (Topping & Luff, 1995). Detergent may, however, reduce the catch of some species of carabid and staphylinid beetle (Pekár, 2002). Pitfall traps can be used to catch natural enemies

whose DNA is to be analysed for determination of diet (Sect. 6.3.12), or in a genetics study. In such cases, it is recommended to test the effect of the trapping fluid/preservative on the DNA of the target natural enemy. Gurdebeke and Maelfait (2002) found that DNA of the spider *Coelotes terrestris* was adequately preserved when 96% ethanol was used as trapping fluid in a funnel pitfall trap (the funnel reduced the evaporation rate of ethanol). RAPD profiles could not be generated from spiders collected in formaldehyde. If pitfall trapping is used as a source of predators for stable isotope analysis (Sect. 6.3.1) traps should be emptied at least every 48 h (Ponsard & Arditi, 2000) because isotopic content changes during decomposition. Skvarla et al. (2014) give an extensive review of the relative merits of different preservatives for pitfall trapping.

It is sometimes necessary, for example when carrying out mark-release-recapture studies (Sect. 6.2.10), to keep specimens alive within the trap. In such cases, it is advisable to place some kind of material in the bottom of the trap to provide a refuge for smaller individuals. Compost, small stones, leaf litter, moss or even polystyrene granules may be used for this purpose. Small pieces of meat may also be added to the traps to reduce cannibalism and interspecific predation (Markgraf & Basedow, 2000), but this may introduce the complication of differential species-specific olfactory attraction (Sect. 6.2.8) which will make the results more difficult to interpret in some types of study. Live-trapping may yield different relative species compositions of predators such as spiders and carabid beetles compared with kill-trapping, and so the two methods are not necessarily comparable (Weeks & McIntyre, 1997).

At the end of a trapping period, it is advisable to replace traps with fresh ones so that the catch can be taken *en masse* back to the laboratory. Plastic pots with snap-on lids are readily available commercially, and are convenient when large numbers of traps need to be transported. To prevent the sides of the hole from collapsing during trap replacement, a rigid liner is useful, and liners can be readily made from sections of plastic drainpipe of an appropriate diameter or by using another plastic cup. In open habitats it is also advisable to place covers over traps in order both to prevent birds from preying on the catch, and to minimise flooding during wet weather. Covers for simple, round traps can be made from inverted plastic plant pot trays, supported by wire. Such covers may increase the catch of carabid and staphylinid beetles that are behaviourally adapted to finding refuge under stones and logs. Soil-coring tools, including bulb planters, can be used to make the initial holes when setting pitfall traps, but it is essential to ensure that the lip of the trap is flush with, or slightly below the soil surface. When traps are operated over a prolonged period of time, some maintenance work is often necessary. For example, in hot, dry weather some soils crack and shrink, creating gaps around the edge of traps, thus reducing their efficiency.

A number of variations on the conventional pitfall trap have been developed in attempts to improve their efficiency or to tailor them for particular habitats and target taxa (reviewed in Skvarla et al., 2014). Lemieux and Lindgren (1999) tested a trap (modified from a design by Nordlander (1987)) with fifteen 1.3 cm-diameter holes around the trap circumference at 2 cm below the upper rim. This trap incorporated a tight-fitting lid and was buried so that the lower 3 mm portion of the holes was below ground level, thus predators could enter the trap through the holes only. The overall catch of carabid beetles was no different from that of conventional pitfalls, but the Nordlander trap had the advantage of excluding vertebrates and reducing the dilution of trapping fluid caused by rainfall. Several workers have used linear traps made from lengths of plastic or metal guttering to increase catches and to obtain directional information on insect movements (Sect. 6.2.11). Sigsgaard et al. (1999) separated two plastic cup pitfalls (positioned back to back) by a barrier of semi-transparent hard plastic sheet to obtain data on directional movement of spiders, carabids and ants entering and leaving a rice field, and a similar design was used by Hossain et al. (2002) to record spiders and carabid beetles moving from harvested to unharvested plots of alfalfa.

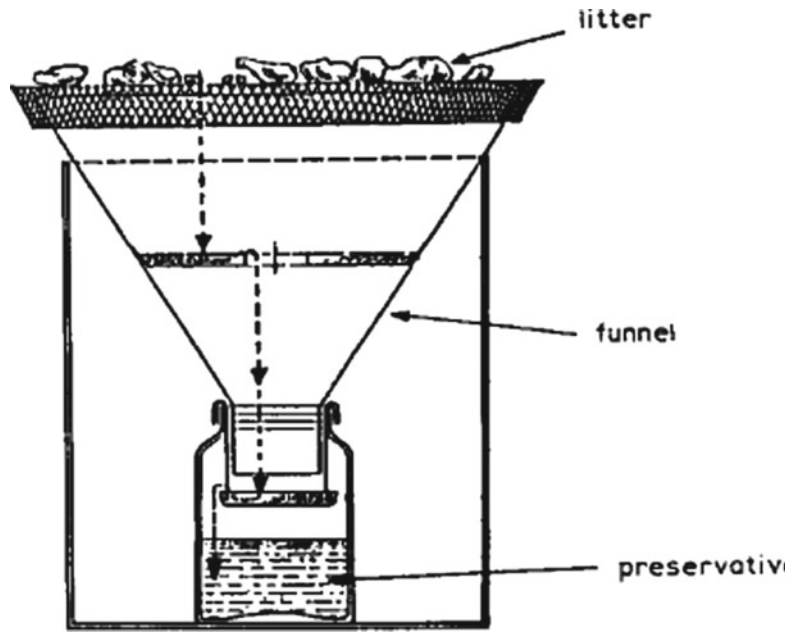

Fig. 6.2 A litter-covered pitfall trap. *Source* Edwards (1991). Reproduced by permission of Elsevier Science

Two-cup pitfalls may be placed a short distance apart and joined by a solid barrier which diverts walking insects into the traps at either end (Wallin, 1985; Jensen et al., 1989; French et al., 2001), or the trap can be placed at the apex of V-shaped fences (Culin & Yeargan, 1983; Markgraf & Basedow, 2000). Winder et al. (2001) found that the use of five traps arranged in a cross formation, and connected by plastic guide barriers (0.5 m lengths of lawn edging sunk 5 cm into the ground), was a more efficient design than single traps for catching some species of carabid and staphylinid beetle, and lycosid spiders (but not linyphiid spiders). Mechanical devices have been used to allow automatic, time-based sorting of trap catches (Williams, 1958; Ayre & Trueman, 1974; Barndt, 1976; Alderweireldt & Desender, 1992; Chapman & Armstrong, 1997; see also Sect. 6.2.11), whilst Heap (1988) suspended UV-emitting, fluorescent light tubes above his traps to increase the catch rate. A funnel trap can be used for sampling in litter, where litter is placed on a gauze mesh across the mouth of the funnel, and predators fall through the mesh and are directed by the funnel into a pitfall trap (Edwards, 1991) (Fig. 6.2). Lund and Turpin (1977) used a funnel pitfall containing liquid nitrogen to freeze carabid beetles as soon as they entered the trap. Immediate freezing prevented predation within the trap and arrested decomposition and enzymatic breakdown of the gut contents of the beetles (which were to be tested serologically (Sect. 6.3.9) for evidence of predation on cutworm larvae). One litre of liquid nitrogen per pitfall lasted about 24 h. A similar funnel pitfall trap (modified to kill entering predators with carbon tetrachloride vapour) was used by Sunderland and Sutton (1980) to collect predators of woodlice in dune grassland. The funnel pitfall of French et al. (2001) contained insecticidal cattle ear tags to kill trapped carabid beetles. Hengeveld (1980) used a funnel trap to direct carabids into a tube containing 4% formalin solution. Luff (1996) considered funnels to be valuable as a way of removing the lip of the pitfall. A funnel was found to prevent carabid beetles hanging onto the trap edge, and it also reduced the likelihood of trapped beetles escaping. Newton and Yeargan (2002) added a varnished hardboard apron surrounding the funnel, and this design was successful for monitoring harvestmen (Opiliones) in soybean, grass and alfalfa crops (see also the experiments of Epstein & Kulman, 1984). Funnel traps were found to be

up to three times more efficient per centimetre of trap diameter, compared to standard cup pitfalls, for catching carabid and staphylinid beetles and lycosid spiders, but no significant improvement in efficiency was reported for ants and linyphiid spiders (Obrist & Duelli, 1996). 'Hanging desk traps' are pitfalls for collecting from rock faces. They have flat collars attached that are hung at a slight angle to the rock face (to prevent rain water flowing in) with the inner edge of the collar taped to the rock and covered with small stones (Růžička & Antušs, 1997). So far, they have been used to assess spider diversity in mountainous areas (Růžička, 2000). Weseloh (1986a) sampled the ground beetle *Calosomasycophanta* on tree trunks using traps that consisted of plastic tree bands that directed climbing beetles into waxed papercups attached to the tree. A similar method, reported by Hanula and Franzreb (1998), utilised an inverted metal funnel attached to the tree trunk. Arthropods, including spiders and harvestmen (Phalangida), crawling up the tree trunk, pass through the funnel and enter a collection container attached to its upturned spout. A 'stalk trap', employing the same principle but on a smaller scale, was used by Lövei and Szentkirályi (1984) to trap carabid beetles ascending and descending the stalks of maize plants. Bostanian et al. (1983) used a 'ramp pitfall' that has two metal ramps leading to a rectangular pitfall, and a metal roof. Several of these traps were run in an untreated carrot field with traditional circular plastic pitfalls running concurrently. The latter caught more carabids plus a range of other invertebrates, but the ramp trap caught 99.5% carabids and was selective, catching the larger species (virtually none <5 mm). This type of trap saves sorting time for researchers interested only in large carabids. The mechanism of size selection is not known, but small species may turn back because of the steepness of the ramp, or be repelled by the darkness. Loreau (1987) devised a pitfall trap to sample adults and larvae of carabid beetles within the soil (Fig. 6.3). A 7 cm-diameter plastic pot is contained within a plastic cylinder which has four 1.5 cm windows at whatever depth it is desired to sample from. The top of the cylinder,

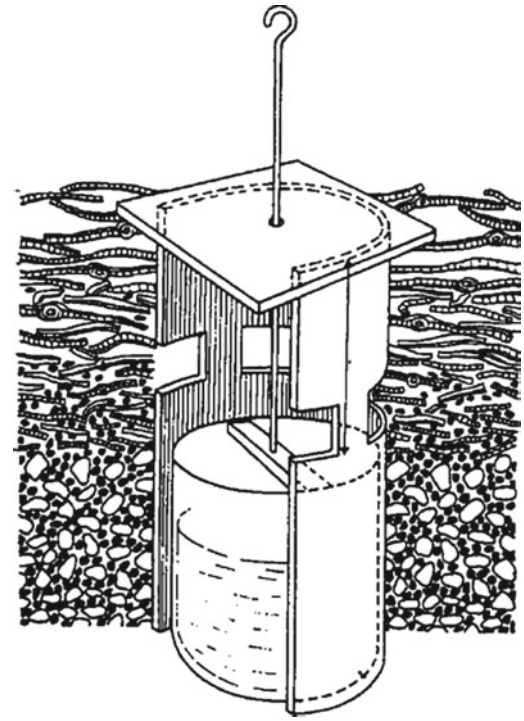

Fig. 6.3 A pitfall trap to study the vertical distribution and activity of adults and larvae of carabid beetles within the soil. *Source* Loreau (1987). Reproduced by permission of Urban and Fischer Verlag

which is enclosed with a plate, is set at the soil surface, or the surface of the litter. Predators enter the cylinder through the windows and fall into the pot which contains preservative. A wire is attached to the pot so that the trap can be emptied without removing the cylinder. Other minor variations in the design of hypogean pitfalls have been reported (Kuschel, 1991; Owen, 1995). Epstein and Kulman (1984) experimented with traps surrounded by plywood aprons, either resting on the rim of the pitfall trap or raised above it, allowing entry by carabids from above or below the apron. Although these trap designs caught fewer beetles than conventional traps, it was shown that trap design can significantly affect both the numbers and ratios of different species caught. Epstein and Kulman suggest that more than one trap design should be used in parallel where activity density of a range of species is being measured. Dormann (2000) designed a trap that can catch predators in

periodically flooded habitats, such as peat bogs and salt marshes. The trap is attached to a slider rod in a tube set vertically in the soil. When the water level rises (e.g., an incoming tide) a Styropor® flotation device below the trap cup pushes the cup up against the roof of the trap, sealing it off from the water (aided by formation of a bell of trapped air). When the water recedes the trap sinks back to soil surface level and capture of walking predators can resume. Traps were not damaged even at times of high tide when there was strong wave action.

Several traps that may be regarded as equivalent to pitfall traps have been developed for sampling aquatic invertebrates (Southwood & Henderson, 2000); that designed by James and Redner (1965) is particularly useful for collecting predatory water beetles. Floating pitfall traps have been devised, and used successfully, to monitor the activity of large wolf spiders of the genus *Pirata* which are adapted for walking on the surface film of water (Renner, 1986).

A common practice for some years has been the erection of physical barriers in the field, usually to enclose defined treatment areas within which pitfall trapping is carried out (Powell et al., 1985; Holopäinen & Varis, 1986) (Fig. 6.4a, b). Caution must be exercised in the use of such barriers in arable crop fields because some predators invade the fields from field boundaries during spring and early summer (Pollard, 1968; Coombes & Sotherton, 1986). The erection of full barriers too early in the year would exclude these species from the enclosed areas, resulting in erroneous data on predator communities. The use of physical barriers can reduce catches of carabid beetles by as much as 35–67% over a growing season (Edwards et al., 1979; De Clercq & Pietraszko, 1983; Holopäinen & Varis, 1986). By contrast, some carabid beetle species emerge as adults from the soil within arable fields (Helenius, 1995) and, if emerging populations are high, many individuals will attempt to disperse away from overcrowded areas. Barriers may prevent this dispersal, resulting in artificially high predator densities within enclosed areas (Powell & Bardner, 1984). Pitfalls inside smaller, replicated fenced and mesh-covered areas ('fenced pitfalls', Sunderland et al., 1995a) can, however, be used to produce a more reliable estimate of predator abundance than would be the case for unfenced pitfalls. The catch over a period of several weeks (Ulber & Wolf-Schwerin, 1995) is assumed to represent a high proportion of those predators that were initially present in the fenced area (where this has been measured for carabid beetles it is usually more than 80%; Bonkowska & Ryszkowski, 1975; Dennison & Hodkinson, 1984; Desender et al., 1985; Holland & Smith, 1997, 1999), but after this period of time the fenced pitfalls need to be moved a few metres (i.e., reset) if the species composition and density of predators are to be continuously monitored (if not moved, they become, effectively, emergence traps, see Sect. 6.2.7). Density estimates from this method compare well with other methods (Gist & Crossley, 1973; Baars, 1979a; Dennison & Hodkinson, 1984; Desender & Maelfait, 1986). Mommertz et al. (1996) compared a variety of sampling methods and concluded that fenced pitfalls should be used for predators in arable crops, but that unfenced pitfalls are a valuable additional method for sampling the larger predators. Holland and Smith (1999) showed a linear relationship between fenced and unfenced traps in the number of six species of carabid beetle caught, but no such relationship for linyphiid spiders. Staphylinid beetles were better represented in fenced than unfenced traps and carabid species composition varied between the two trap types, with smaller species being more evident in the fenced traps (Holland & Smith, 1999). Fences or isolators are usually constructed of wood (Desender & Maelfait, 1983; Dennison & Hodkinson, 1984; Holland & Smith, 1999), metal (Bonkowska & Ryszkowski, 1975; Grégoire-Wibo, 1983a, b; Scheller, 1984; Helenius, 1995) or plastic (Sunderland et al., 1987b), and the mesh covering needs to be very tightly sealed because small climbing predators can be attracted to the traps and enter through small gaps,thus inflating the density estimate (Sunderland et al., 1995a). Fenced pitfalls have been used to estimate the density of carabid beetles (Basedow, 1973; Bonkowska & Ryszkowski, 1975; Baars, 1979a; Dennison & Hodkinson, 1984; Desender

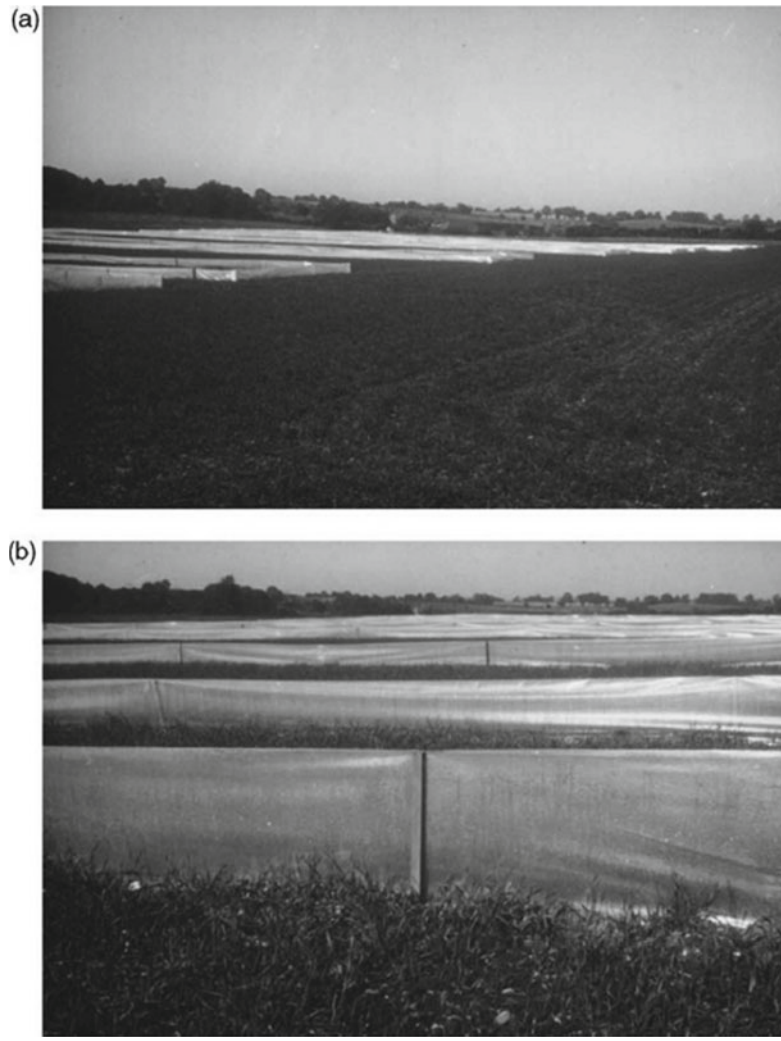

Fig. 6.4 **a** Polyethylene barriers surrounding experimental plots containing pitfall traps to restrict immigration by carabid beetles. **b** Close-up of polyethylene barriers

& Maelfait, 1986; Helenius, 1995; Ulber & Wolf-Schwerin, 1995), staphylinid beetles, and spiders (Basedow & Rzehak, 1988). Connecting the pitfalls with small barriers within the fenced area will increase capture efficiency (Durkis & Reeves, 1982; Ulber & Wolf-Schwerin, 1995; Fig. 6.5). Further aspects of pitfall trap methodology are discussed in Luff (1975, 1987), Adis (1979), Scheller (1984).

Pitfall trap catches are a function of predator abundance, activity and trappability (= probability of capture of an individual in the population, Melbourne, 1999), and so changes in any of these variables will affect the capture rate. Locomotor activity in natural enemies and methods for its investigation are discussed in Sect. 6.2.11. Thomas et al. (1998) recorded four peaks in pitfall catch of the carabid *Pterostichusmelanarius* during a period when density (estimated by mark-release-recapture, see Sect. 6.2.10) showed a smooth increase to a plateau. The peaks in pitfall catch were correlated with periods of rainfall. The number of individuals present in a trap at the end of a trapping period is determined by both the capture rate (the rate at which individuals fall into the trap) and by the escape rate (the rate at which individuals manage to escape from the trap). Both capture

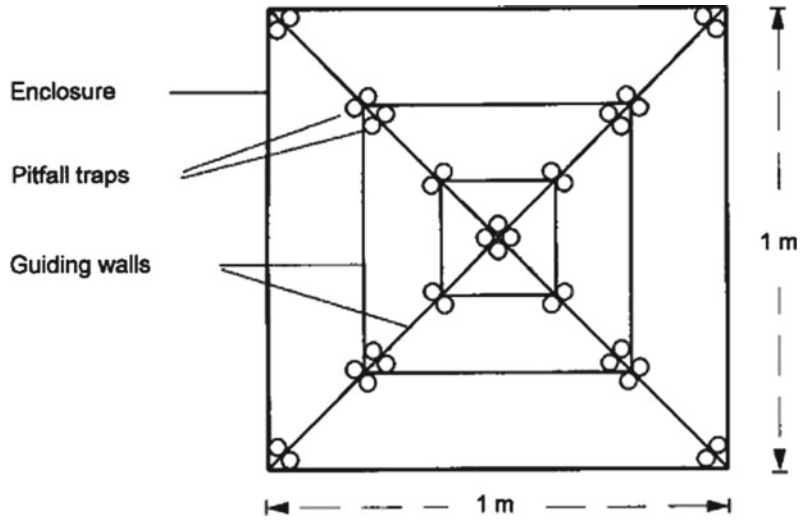

Fig. 6.5 Fenced pitfall trapping comprising an enclosure with 36 pitfall traps and guiding walls. *Source* Ulber and Wolf-Schwerin (1995). Reproduced by permission of Aarhus University Press

rate and escape rate depend on the predator species (and its sex and hunger level) and they are also influenced by a variety of factors including the size, shape, pattern and spacing of trap, presence of a trap cover, type of preservative used, vegetation density, soil type, soil surface texture, food availability and weather conditions (Heydemann, 1962; Luff, 1975; Uetz & Unzicker, 1976; Thomas & Sleeper, 1977; Adis, 1979; Müller, 1984; Niemelä et al., 1986; Benest, 1989; Abensperg-Traun & Steven, 1995; Work et al., 2002). In a mowed weedy field in the Czech Republic the average increase in catch of carabids per 1 °C increase in temperature was 6.3% (Honěk, 1997).

There is also evidence that some beetles aggregate in pitfall traps (Thomas & Sleeper, 1977; Benest, 1989), probably in response to aggregation or sex pheromones or to defensive secretions, and this can result in considerable inter-trap variability in catches (Luff, 1986). Raworth and Choi (2001), however, found no significant attraction of the carabid *Pterostichus melanarius* to traps that already contained this species, but a maximum of three 'bait' individuals were used, and responses to higher densities remain to be investigated. Penagos et al. (2003) considered that male carabids (*Calosomacalidum*) were attracted to traps containing a female and that trapping results were affected by this sexual attraction. Inter-trap variability is also affected by weeds, ants, and carcasses of small mammals that have fallen into the trap, soil type and the pH of the topsoil (Törmälä, 1982; Honěk, 1988; Powell et al., 1995). All of these influences should be considered when interpreting pitfall trap data.

Pitfall traps do not provide absolute measures of predator density because the number of individuals caught depends partly on their locomotor activity. Therefore, the abundance of a species as measured by pitfall trap catches has been termed its activity density or activity abundance (Heydemann, 1953; Tretzel, 1955; Thiele, 1977). The activity density of a species may provide, to some extent, a measure of the predator's role in an ecosystem (for example in capturing prey), since this role sometimes depends on its mobility as well as on its frequency (Thiele, 1977). Considerable caution is, however, needed here, because some predators have a 'sit-and-wait' foraging strategy, and intense predator activity can be associated with the search for mates rather than for food, or even with the need to escape from an inimical environment. Exclusive reliance on pitfall trapping in ecotoxicological studies is inadvisable because pesticides can directly increase (Jepson et al., 1987) or decrease (Everts et al., 1991) activity, or indirectly affect activity by altering food availability (Chiverton, 1984).

In some cases (e.g., carabid beetles) hungry predators have been shown to be more active than satiated predators (Mols, 1987), and the relationship between degree of hunger and activity level (and, thus probability of capture) can vary significantly according to habitat type (Fournier & Loreau, 2000). Locomotor activity, and therefore capture rate, often varies between the two sexes of a species, so that reliable estimates of sex ratio within predator populations are also difficult to obtain from pitfall data. For example, most of the spiders which are active on the ground in cereal crops are males, and so more males than females are caught in pitfall traps (Sunderland, 1987; Topping & Sunderland, 1992). As a confounding factor, there is also good evidence to suggest that sex ratios of some carabids caught in traps will vary according to the state of nutrition of the predators (Szyszko et al., 1996). Females may be less active than males when well fed, because both sexes spend less time searching for food but the males continue to search for females. When hungry, females may be more active than males because they have a greater food requirement related to egg production.

Pitfall traps have been used to compare activity densities for the same species in different habitats and at different times of year but it must be remembered that the activity densities of different species are not necessarily comparable (Bombosch, 1962). It is difficult to separate the influences of activity and abundance on trap catches, but, when trapping is continued throughout the year, whole-year catches may be linearly related to density for some individual species (Kowalski, 1976; Baars, 1979a). Using this 'annual activity' approach, analyses are restricted to within-species and within-habitat comparisons, (not within-season) and are also not appropriate for all species. Loreau (1984a), for example, showed that the annual activity of the carabid *Pterostichusoblongopunctatus* was not linearly related to its population density. The 'within-habitat' restriction was emphasised by a study of pitfall catches of ants in grassland, where an area of 80 cm radius centred on each trap was either unmodified, cleared of litter, or cleared of all vegetation (Melbourne, 1999). These habitat manipulations had a statistically significant effect on abundance, relative abundance, species richness and species composition of the catch. Trappability increased, overall, as the habitat became more open, but there was considerable interspecific variation in response to degree of clearing (Melbourne, 1999). If sufficient data are available for a particular species of natural enemy, it may be possible to estimate population density from pitfall catches. Raworth and Choi (2001) measured the rate and direction of movement of the carabid *Pterostichus melanarius* in relation to temperature in the laboratory. They used these data in a simulation model of beetle movement which was then calibrated and validated using mark-release-recapture during July in a grid of pitfall traps in a raspberry field (which had a flat surface and few weeds, and so was in this respect similar to laboratory arenas). The model showed that pitfall catch should increase linearly with beetle density, with the slope of the relationship being temperature dependent. The authors stress that the equations and data are restricted to *P. melanarius* under the specified conditions, but this approach for converting pitfall catch to density estimates could be applied to other species and habitats, if the considerable amount of work entailed were justified in particular cases.

It is difficult to obtain from pitfall trap catches an accurate picture of the relative abundance of different species within a community, because different species are caught at different rates and also escape at different rates (Jarošsik, 1992; Topping, 1993; Lang, 2000). Mommertz et al. (1996) found that even carabid beetles of the same size and genus (*Poecilus*) exhibited large differences in trappability in an artificial wheat field in the laboratory. Video-recording techniques in the laboratory (Halsall & Wratten, 1988a) and the field (N. Paling, personal communication) have also been used to show that predator species vary greatly in trappability and the capacity to escape from pitfalls. Jarošík (1992) attempted to use pitfall trap data to

compare patterns of species abundance in communities of carabid beetles from different habitats, but concluded that pitfall trap data were inadequate for this purpose. Pitfall trapping in unfenced areas usually gives very different results to other methods (such as D-vac, photoeclectors, ground search in quadrats, soil flotation, fenced pitfalls) for the species composition, size distribution, sex ratio and relative abundance of polyphagous predators (Bonkowska & Ryszkowski, 1975; Lohse, 1981; Desender & Maelfait, 1983, 1986; Dennison & Hodkinson, 1984; Basedow & Rzehak, 1988; Dinter & Poehling, 1992; Topping & Sunderland, 1992; Andersen, 1995; Dinter, 1995; Ulber & Wolf-Schwerin, 1995; Lang, 2000; Arneberg & Andersen, 2003). For example, Lang (2000) found that the abundances of carabid beetles and lycosid spiders were overestimated by pitfalls, but the abundances of staphylinid beetles and linyphiid spiders were underestimated, compared to catches made with photoeclectors (Sect. 6.2.7). Pitfalls have sometimes been used in the study of ant communities. Lobry de Bruyn (1999) reviews the utility and limitations of pitfalls for estimating ant biodiversity. Pitfall trapping does provide useful presence/absence data, and could complement other collection methods in estimating the natural enemy biodiversity of a particular region. For example, Wesolowska and Russell-Smith (2000) caught 44 species of jumping spider (Salticidae) in pitfall traps in a variety of habitat types in Mkomazi Game Reserve (Tanzania). This compared with 34 species caught by hand and 15 species by sweep netting. Approximately half of the species collected by each method were collected by that method alone, and each method collected a similar proportion of rare species. Species lists derived from extensive trapping programmes can be used, with the aid of modern ordination techniques (Pielou, 1984; Digby & Kempton, 1987), to classify different habitats based on their carabid communities, or to identify environmental factors which are influencing species distributions (Eyre & Luff, 1990; Eyre et al., 1990). Large-scale trapping programmes can entail the commitment of a large amount of resources and it becomes necessary to optimise the use of these resources. For spiders in an agricultural landscape, Riecken (1999) found that using fewer traps resulted in less of a reduction in the total number of species caught than did reducing the duration of sampling.

With the addition of appropriate analytical techniques, pitfall trapping programmes can be used to estimate the biodiversity of a group of predators in a particular habitat. Samu and Lövei (1995), for example, used pitfalls to collect ground-dwelling spiders in a Hungarian apple orchard. Simulation of increased sample size was made by computer sub-sampling of the original data set. An asymptotic function (taken from the theory of island biogeography) was used to describe the sampling curve and the value for the asymptote was considered to be a realistic estimate of the total number of species present. Brose (2002) showed that sampling effort can be reduced if carefully selected non-parametric estimators are used to calculate the species richness that would be obtained from a more intensive pitfall sampling programme. It should be remembered, however, that it is likely that some species of the natural enemy group(s) of interest will not be amenable to capture in pitfall traps. Estimating species richness of invertebrate groups that are active both on the ground and in vegetation requires multiple sampling techniques, such as pitfall trapping combined with suction sampling or sweeping (Standen, 2000).

Information on the spatial dispersion patterns and density of predators living on the soil surface can be obtained using pitfall traps either spaced in a grid system (Ericson, 1978; Gordon & McKinlay, 1986; Niemelä, 1990; Bohan et al., 2000), laid out as transects through heterogeneous habitats (Wallin, 1985), or as concentric circles at fixed distances from a central point (Parmenter & MacMahon, 1989; Buckland et al., 1993). Traps within a regular grid system may interfere with each other, the central traps catching fewer individuals than the outer traps (Scheller, 1984). Trap spacing is therefore important, interference increasing as between-trap distances decrease. In Canadian conifer forests it was determined that traps needed to be

at least 25 m apart to reduce depletion effects (Digweed et al., 1995). Measurement of the spatial aggregation of invertebrates may be radically affected by between-trap distances within a sampling grid. Bohan et al. (2000) found that, at 4 and 8 m scales, pitfall trap catches of the carabid *Pterostichus melanarius* were randomly distributed, but at a 16 m scale catches were spatially aggregated. The scales at which aggregation occurs are likely to be very different between species and habitats and will change over time. The catch may also be increased if the surrounding ground is disturbed during trap establishment ('digging-in effects') (Digweed et al., 1995). Similarly, within heterogeneous habitats, differences in vegetation type and density around traps will affect capture rates, hindering the investigation of dispersion patterns. Consideration also needs to be given to aggregation that may occur in response to infochemicals (Chap. 1). Such aggregation may be independent of trap positions and can vary with between-trapping periods (Luff, 1986).

Despite their limitations, pitfall traps remain a very useful sampling tool for obtaining both qualitative and quantitative data on those predators which are active on the soil surface, providing that the objectives of the sampling programme are clearly defined and that the many factors which can influence the catch rate are considered during data interpretation. Advice on the interpretation of pitfall data is given by Luff (1975, 1987), Adis (1979), Ericson (1979), Baars (1979a). For example, if trapping is carried out over a long period of time then the data collected can be representative of actual abundance (e.g., Gist & Crossley, 1973; Baars, 1979a). Despite criticisms (Southwood, 1966), pitfall trapping methods are probably the most often used to compare epigeal communities (e.g., Rich et al., 2013; Zmihorski et al., 2013; Wang et al., 2014). However, some small-bodied natural enemies live on or in leaf litter (e.g., some diapriid and eucoilid parasitoids) and pitfall trapping is unlikely to be useful in sampling such insects: a leaf litter sampling technique (Sect. 6.2.7, and Southwood & Henderson, 2000) might be more appropriate.

6.2.2 Vacuum Netting

Although several different types of vacuum net have been developed, nearly all operate on the same principle. They employ a fine-mesh net enclosed in a rigid sampling head which is attached to a flexible tube. The tube is connected to a fan which is driven by an electric or petrol-fuelled motor. The fan draws air through the flexible tube via the net in the sampling head, sucking small arthropods on to the net from the vegetation enclosed by the sampling head. The sampling tube and its head are quite wide (sampling approximately 0.1 m^2) in some machines (Dietrick et al., 1959; Dietrick, 1961; Thornhill, 1978; Duffey, 1980) (Fig. 6.6a), but narrower (approximately 0.01 m^2) in others (Johnson et al., 1957; Heikinheimo & Raatikainen, 1962; Southwood & Pleasance, 1962; Arnold et al., 1973; Henderson & Whitaker, 1976; Macleod et al., 1995) (Fig. 6.6b). Machines with wide tubes require a more powerful motor in order to attain the required air speed of at least 90 km/h through the collecting head. Many of the machines used in the UK are of the wide-tube variety and are driven by two-stroke, lawnmower, petrol-fuelled engines which are mounted on rucksack frames so that they can be carried on the back of the operator (Thornhill, 1978). Taubert and Hertl (1985) designed a small vacuum net driven by a battery-powered electric motor, presenting a lighter load for the operator. New designs of vacuum insect net continue to be devised and evaluated (Summers et al., 1984; Holtkamp & Thompson, 1985; De Barro, 1991; Wright & Stewart, 1992; Macleod et al., 1994, 1995; Samu & Sárospataki, 1995a; Toft et al., 1995). The modified Allen-vac has a collecting bag held sufficiently far inside the apparatus that it does not become snagged in dense woody scrub or desert thorn scrub (Osborne & Allen, 1999). The corn KISS (keep-it-simple-sampler) (Beerwinkle et al., 1999), is a hand-held modified leaf blower that directs air across a maize plant and into a net, which is also attached to the blower (i.e., this is a blower rather than a vacuum device, and the plant is sandwiched between

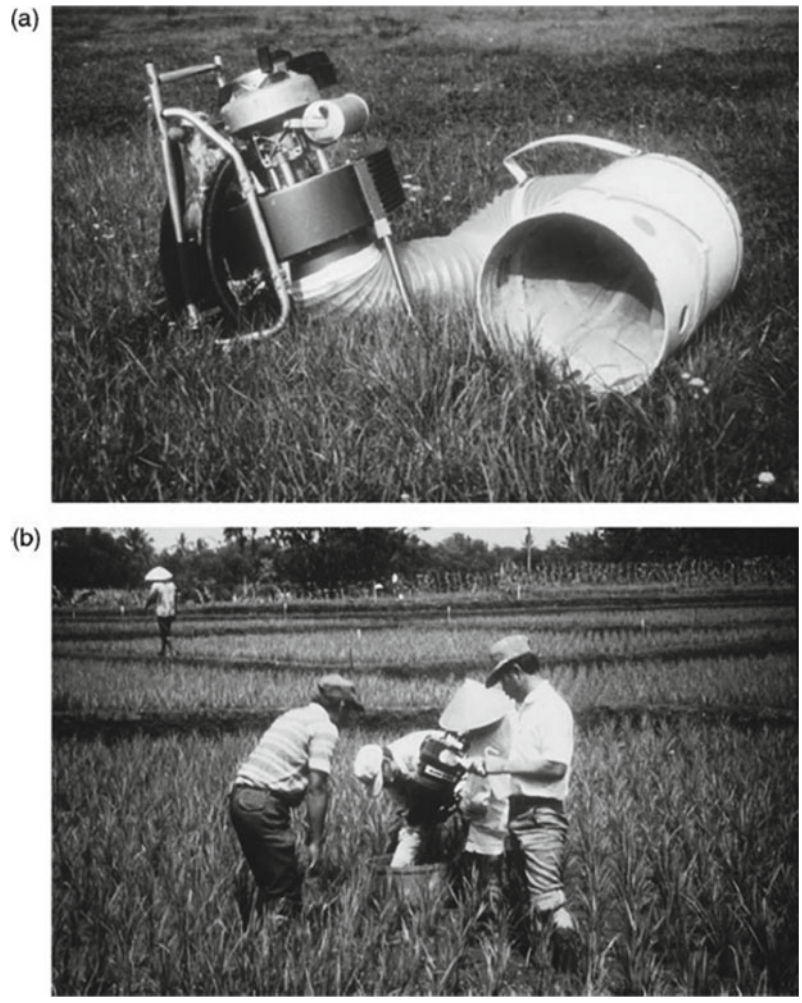

Fig. 6.6 Vacuum sampling. **a** Wide-nozzled (0.1 m^2), petrol engine-driven vacuum sampler; **b** Entomologists vacuum sampling rice insects in a paddy in Indonesia. Reproduced by permission of Anja Steenkiste

blower and net). To sample a plant the KISS is lifted from the base to the top of the plant in a sweeping motion. Beerwinkle et al. (1999) found that the KISS was as efficient as Berlese extraction (Sect. 6.2.7) for sampling mobile predators (such as spiders, ladybird larvae, nymphal predatory bugs, and lacewing larvae) exposed on the plant surface. The aquatic equivalent of this device is the 'air-lift sampler', which blows air at the substrate and forces dislodged insects into a mesh cup (Williams & Feltmate, 1992).

Vacuum nets can be used in a number of different ways to collect arthropods (natural enemies or hosts and prey) from vegetation. A commonly used method involves pressing the sampling head to the ground over the vegetation (providing this is not too tall or dense) and holding it in place for a pre-defined period of time (e.g., 10 s), a process which may be repeated several times within a specified area to form a single sample. This method is appropriate for wide-nozzled machines, and, by measuring the size of the sampling head, the catch can be related to a finite area of vegetation, so giving an absolute measure of species densities.

An alternative method of using a wide-nozzled suction sampler is to hold the sampling head at an angle to the ground whilst pushing it through the vegetation over a defined distance. As it brushes through the vegetation the advancing sampling head dislodges arthropods which are then sucked into the net. This method

is particularly useful when collecting insects from crops planted in discrete rows as it allows a specified length of row to be sampled. Graham et al. (1984), however, found that placing the sampling head vertically over plants was more efficient than sweeping it through the vegetation for sampling parasitoid wasps (Mymaridae) and predatory heteropteran bugs (Nabidae) in alfalfa. Motorised vacuum nets have also been used to sample natural enemies from the foliage of fruit trees (Suckling et al., 1996; Green, 1999; Gurr et al., 1999).

Another approach involves enclosing an area of vegetation with a bottomless box or cylinder and using a narrow-nozzled sampler to remove insects from the enclosed vegetation, the soil surface and the interior walls of the box (Johnson et al., 1957; Southwood & Pleasance, 1962; Henderson & Whitaker, 1976; Smith et al., 1976; Törmälä, 1982; Summers et al., 1984; Heong et al., 1991; Wright & Stewart, 1992; Toft et al., 1995; Schoenly et al., 1996a; De Kraker et al., 1999). If the vegetation is tall, it can then be cut and removed before sampling a second time. Wright and Stewart (1992) adapted a commercial garden leaf blower (Atco Blow-Vac) as a narrow-nozzled suction sampler and used it to extract insects from areas of grassland enclosed by an acetate sheet cylinder. Compared with a wide-nozzled suction sampler, the sampling head of which covered the same area of ground as the acetate cylinder, the narrow-nozzled apparatus extracted significantly more predatory beetles and spiders from the vegetation. Haas (1980) used repeated suction sampling within a gauze-covered isolator in grassland, and Marston et al. (1982) used a D-vac to remove invertebrates after knock-down by permethrin inside cages in a soybean crop. Highly active species may escape from the sampling area when the sampler approaches, and researchers have devised a number of ingenious modifications of technique in an attempt to circumvent this problem. These range from a cylindrical cage on the end of a pole that can be dropped over a sugar beet plant from a distance, before vacuum sampling the enclosed area (Hills, 1933), to various more sophisticated 'drop trap' methods operating on the same basic principle (Turnbull & Nicholls, 1966; Gromadzka & Trojan, 1967; Mason & Blocker, 1973). Duelli et al. (1999) took suction samples from the inside of a cubic tent placed over the vegetation. They found that by quickly tilting the cube over the vegetation against the wind they could trap most predators inside the tent, including fast-flying adult flies. The problem of large, active predators fleeing from an area to be sampled due to the noise and vibration of the approaching researchers (Uetz & Unzicker, 1976; Samu & Kiss, 1997) is common to most of the field sampling techniques described in this chapter, and 'drop trap' methods could be more widely considered. Other modifications to suction sampling techniques include adjuster bars to enable sampling at various vertical distances above ground (Kennedy et al., 1986), extension tubes to reduce mortality of natural enemies in the collecting net (Topping & Sunderland, 1994), and a flange within the D-vac head to reduce escape rates of large predators (Yeargan & Cothran, 1974).

After each sample is taken with a vacuum net device, the collecting net is usually removed from the sampling head. The net can either be tied off (to secure the catch) and replaced with a fresh one, or the catch can be transferred to a polythene bag so that the net can be re-used. Moreby (1991) described a simple modification to the net and sampling head that speeds up the transfer of samples to polythene bags. In order to reduce the risk of losses due to predation within the net or bag, the catch should be killed whilst still in the field. This can be done by placing the tied-off nets into bags already containing a chemical killing agent or by adding wads of cloth or paper, impregnated with killing agent, to the catch once it has been placed in the polythene bag. For some purposes however, such as the rearing of parasitoids from hosts (Sect. 6.3.6), the catch may need to be kept alive.

On returning to the laboratory, catches may be sorted by hand but it is often useful to pass the sample through a series of sieves before hand-sorting, especially if organisms of a fixed size are being counted. Hand-sorting can be extremely time-consuming, particularly if very small insects

Fig. 6.7 Contents of a vacuum sample taken in a cereal field in July in southern England. The catch shown is the result of five 10-s samples, each taken from an area of 0.1 m^2. The catch is preserved in 70% ethanol and is divided into Hemiptera (mainly aphids), Coleoptera (including larvae), Diptera, Hymenoptera 'Parasitica', spiders and soil and plant debris

are being sampled (Fig. 6.7). Consequently, some workers have used Berlese funnels or flotation techniques to speed up the process (Dietrick et al., 1959; Marston, 1980). Further details on the construction of vacuum nets and on their use may be found in: Johnson et al. (1957), Dietrick et al. (1959), Dietrick (1961), Southwood and Pleasance (1962), Weekman and Ball (1963), Arnold et al. (1973), Thornhill (1978), Kogan and Herzog (1980), Southwood and Henderson (2000).

The efficiency of vacuum nets varies considerably in relation to the biomass (Bayon et al., 1983) and vertical stratification (Sunderland & Topping, 1995) of the natural enemies being sampled, and the height, density and type of vegetation being sampled (Henderson & Whitaker, 1976). Dense vegetation may affect efficiency by reducing airflow and by forming a mat under which natural enemies can cling and avoid capture. This problem can be reduced by taking a suction sample, then immediately cutting the vegetation to a low height and re-sampling (Dinter, 1995; Hossain et al., 1999). When this protocol was applied to lucerne, recapture rates of marked ladybirds and predatory heteropterans were increased from approximately 0.7–0.9 (Hossain et al., 1999). Fenced pitfall traps (Sect. 6.2.1) can be a useful alternative to suction sampling for estimating the density of large carabid beetles, because these traps are appropriate for nocturnal as well as diurnal species and are less affected than vacuum nets by the density of vegetation in the sampled habitat (Holland & Smith, 1999). Generally, small, winged insects such as Diptera and adult Hymenoptera 'Parasitica' are sampled with the greatest efficiency by suction sampling, and Poehling (1987), working in cereal crops, concluded that it is a suitable method for sampling these types of insect. An investigation was made (W. Powell, unpublished data) into the efficiency of a wide-tubed vacuum net (area of sampling head: 0.1 m^2) for sampling adult parasitoids in flowering winter wheat. Known numbers of parasitoids were released into large field cages (9 m × 9 m × 6 m) and

allowed to settle for several hours before sampling. Comparisons of expected and actual catches indicated a sampling efficiency of over 90%. Henderson and Whitaker (1976) investigated the efficiency of a narrow-tubed vacuum net used in conjunction with a bottomless box which enclosed a 0.5 m^2 area of grassland. Again, efficiency was highest for Diptera (79–98%) and Hymenoptera (60–83%) and was lowest for Acarina (12–40%). Sampling efficiency tended to decline with increasing grass height for most of the groups sampled. Vacuum nets are also regarded as an efficient means of sampling adult parasitoids in soybean crops (Marston, 1980). Duffey (1974) found the efficiency of the D-vac to be only 14–58% for spiders in pasture. He also suspected that invertebrates were sucked into the nozzle from beyond the 0.09 m^2 nozzle area. This was confirmed by Samu et al. (1997), who caught three times more spiders in an alfalfa transect of 48 vacuum insect net subsamples compared with suction sampling an enclosed part of the crop of the same area as the transect. This lateral suction effect can be corrected by mathematical conversion (Pruess et al., 1977) or by sampling within small enclosures (see above). The efficiency of a 'Blower-vac' suction sampler was 60–70% for pests and natural enemies in apple trees, when compared with destructive sampling. This was superior to the use of a beating tray which failed to detect half the species present, but *Orius* bugs were underrepresented (Suckling et al., 1996).

The time of day when samples are taken can also influence vacuum net catches, because the locomotor activity of most natural enemies varies during the day. Vickerman and Sunderland (1975) used a vacuum net and sweep nets to compare the diurnal and nocturnal activity of predators in cereal crops. More adults and larvae of staphylinid beetles (*Tachyporus* spp.) were caught during the hours of darkness than in the daytime, as were spiders, hover fly larvae and earwigs (*Forficula auricularia*). Whitcomb (1980) used a vacuum net to sample spiders in soybean fields, but Sunderland (1987) advocated a combination of vacuum netting and ground searching to estimate spider densities in crops, because some species are more accurately assessed by the latter technique than by the former, whereas the reverse is true for other species. More robust predators, such as adult beetles, and those which can rapidly move out of the way of the advancing operator, are less efficiently sampled by a vacuum net (Sunderland et al., 1987b). Green (1999) compared spider diversity from diurnal and nocturnal vacuum net sampling of citrus trees with that from continuous pitfall sampling of the ground below the trees. Green concluded that a combination of sampling methods and timings were vital to avoid a biased interpretation of the composition of spider assemblages.

Because of the variability in sampling efficiency in relation to different natural enemy groups, vacuum netting is not always useful for comparing the relative abundances of different taxa in communities. When using a vacuum net in a sampling programme it is important, whenever possible, to calibrate the data collected by comparing them with data obtained using an absolute sampling method (Smith et al., 1976; Pruess et al., 1977; Whitcomb, 1980; Dewar et al., 1982; Dinter & Poehling, 1992; Topping & Sunderland, 1994; Dinter, 1995; Sunderland & Topping, 1995). Usually, once this is done, the vacuum net can be used to obtain absolute estimates of predator population densities by sampling discrete units of vegetation. A series of samples taken in a regular grid pattern can then be used to investigate the spatial dispersion pattern of individual natural enemy species.

A further limitation on the use of vacuum nets is that moisture on the vegetation being sampled severely reduces net efficiency, and so use either during wet weather or following a heavy dew is not advised, since large numbers of insects tend to adhere to the sides of the net and to the inner surfaces of the collecting head (Törmälä, 1982). Not only does this make their efficient removal from the net difficult but delicate arthropods (e.g., aphids, leafhoppers, linyphiid spiders) can be damaged, hampering their identification to sex or species. De Barro (1991) claimed that wet vegetation becomes less of a problem if a Blower/Vac sampler with a high nozzle air

velocity that expels free water through the blower tube is used. Schoenly et al. (1996a) sampled from irrigated rice fields using a suction device (vacuuming 0.8 m^3 air min^{-1}) that delivered arthropods and water through a hose into a plastic reservoir containing a nylon mesh strainer. Ethanol was then used to wash the collected arthropods (including parasitoids, spiders, and predatory bugs) from the strainer into a collecting tube.

A lightweight (7.8 kg), petrol-engined vacuum sampler without a net ('Vortis', manufactured by the Burkard Manufacturing Company Ltd, of Rickmansworth, UK, http://www.burkard.co.uk/homepage.htm) operates on a different principle from many other samplers. Air, instead of being drawn in through the sampling nozzle, enters above ground level. Static vanes above the air inlet create a vortex, lifting insects into an expansion chamber, from where they are deposited into a detachable, transparent collecting vessel outside the apparatus. As well as being lightweight and therefore very portable, the device has several advantages over vacuuum nets: (1) Suction pressure remains constant even after many hours of operation. Because of the way the insects are accumulated, a fall-off in suction pressure does not occur (the nets in other devices need to be repeatedly unclogged of accumulated insects and debris in order to avoid a reduction in suction pressure). (2) Because of the type of suction mechanism the sampler employs, the nozzle can be applied continuously to the ground during sampling, thus saving time (with vacuum nets the nozzle has to be repeatedly lifted from the ground during sampling, to allow the entry of air). The Vortis, although regarded as superior and less expensive than the D-vac, is still fairly dear and, despite the vortex effect, still suffers a reduction in suction power when used for an extended period. Much cheaper, and equally, if not more efficient suction samplers, can be 'manufactured' by reverse engineering standard garden leaf blowers, the 'G-vacs' (e.g., Zentane et al., 2016).

Vacuum sampling of natural enemies from the air has also been carried out (see suction traps in Sect. 6.2.11).

6.2.3 Sweep Netting

A sweep net comprises a fine-meshed, cone-shaped net mounted on a rigid, circular, five-sided, or D-shaped frame which is attached to a short handle. It is commonly used for collecting arthropods from vegetation (especially herbaceous vegetation) because it is inexpensive, highly portable and easy to use, and also because it allows the rapid collection of a large number of insects. Sweeping is a method particularly suitable for the collection of small hymenopteran parasitoids, especially chalcidoids, proctotrupoids, cynipoids and braconids (Noyes, 1989), and a sweep net has been designed specifically for this purpose (see Noyes, 1982, for details).

As the name suggests, sampling with the net involves sweeping it rapidly through the vegetation so that the rigid frame dislodges insects that are then caught in the moving net. It may be either swept backwards and forwards in a simple arc or made to follow a more sinuous track, for example a figure-of-eight. Each sampling will normally consist of a fixed number of sweeping movements of fixed speed and fixed duration over a predetermined path through the vegetation. After the sample has been taken, less active natural enemies may be selectively extracted from the net using an insect aspirator (pooter). Alternatively, if the species being collected are active flyers, the sample can be transferred to a polythene bag and extracted later in the laboratory. As with vacuum net sampling, it is advisable to kill the catch immediately after capture to prevent losses from predation, unless the insects are specifically required to be kept alive.

It is important, however, to define a standard sweeping technique before commencing a sampling programme, because both the method and the pattern of sweeping can significantly affect capture rates (DeLong, 1932; Kogan & Pitre, 1980; Gauld & Bolton, 1988). For example, Gauld and Bolton (1988) point out that with hymenopterans in the Parasitica the particular sweeping technique used can account for as much as a 20-fold difference in catch size and a corresponding difference in the diversity of wasps

caught. It is advisable to practice one's chosen technique in order to achieve a reasonable level of consistency.

Although they are frequently used for sampling arthropods in crop fields, sweep nets are subject to considerable sampling variability because their efficiency is affected by a range of factors. These include the distribution, density, activity and life stage of the organism being sampled (Ellington et al., 1984) as well as vegetation height and density, and climatic conditions (Southwood & Henderson, 2000). In addition, the proficiency of different operators often varies significantly. It is also very difficult to relate a sweep net sample to a finite unit or area or volume of vegetation, making absolute estimates of population densities almost impossible to obtain using this method alone. There is also considerable variation in the area of the net itself as different suppliers offer a range of net types. Nevertheless, Tonkyn (1980) developed a formula which he used to express sweep net data as the number of insects caught per unit volume of vegetation sampled, thereby facilitating comparisons with other sampling methods.

Sedivy and Kocourek (1988), studying variation in the species composition of herbivore, predator and parasitoid communities in alfalfa crops, compared the sampling efficiency of a sweep net with that of a vacuum net. They concluded that larval lacewings (Chrysopidae) were caught equally well by both methods, but that the sweep net was more efficient in capturing adult ladybirds (Coccinellidae) and adult hover flies (Syrphidae), whereas the vacuum net was more efficient at capturing adult lacewings, nabid bugs and parasitoid wasps. The same two methods have also been compared in relation to the sampling of spiders in soybean fields, where 34% fewer spiders were collected by the sweep net than by the vacuum net (LeSar & Unzicker, 1978). In cereal crops, Poehling (1987) found the sweep net to be more suitable than the vacuum net for sampling ladybird and hover fly larvae. As with vacuum nets, different natural enemy species are caught with different efficiencies by the sweep net, and the time of day when sampling is carried out affects sweep net catches in the same way as it affects vacuum net catches (Vickerman & Sunderland, 1975) (Sect. 6.2.2). Wilson and Gutierrez (1980) assessed the efficiency of a sweep net in the sampling of predators in cotton, comparing this method with visual counting carried out on whole plants. The sweep net was only 12% efficient in estimating total predator numbers compared with the visual counts. When individual species were considered, the sweep net was the more efficient method only for detecting lacewing adults, because these insects were easily disturbed and tended to fly away during visual counting. In addition, the vertical distribution of predators on the cotton plants affected the efficiency of the sweep net, which was most efficient for catching those species with a distribution biased towards the top of the crop canopy. Fleischer et al. (1985) likewise concluded that the sweep net is an inefficient method for sampling predators in cotton. Sweeping was found to be inappropriate for sampling taxa (such as Gerridae and Lycosidae) that are normally found at the base of rice hills (Fowler, 1987). Elliott and Michels (1997) developed regression models to convert sweep net data into density estimates for ladybirds in alfalfa. When plant height was included in the model, the coefficient of determination of the regression for estimating adult density was 0.93, and the authors concluded that sweep netting was an efficient method for estimating ladybird densities in alfalfa IPM programmes.

Mayse et al. (1978) compared sweep netting with direct observation and the use of a 'clam trap' for sampling arthropods in soybean. The trap is a 30 cm × 90 cm hinged wooden frame supporting a cloth or organdy bag open on three sides (Fig. 6.8). It is brought down rapidly over a row of soybean plants and clamped shut with latches. The plants are then cut through at ground level, after which the organdy bag (containing plants and arthropods) can be transferred to a plastic bag for transport to the laboratory, where the arthropods are extracted by washing with

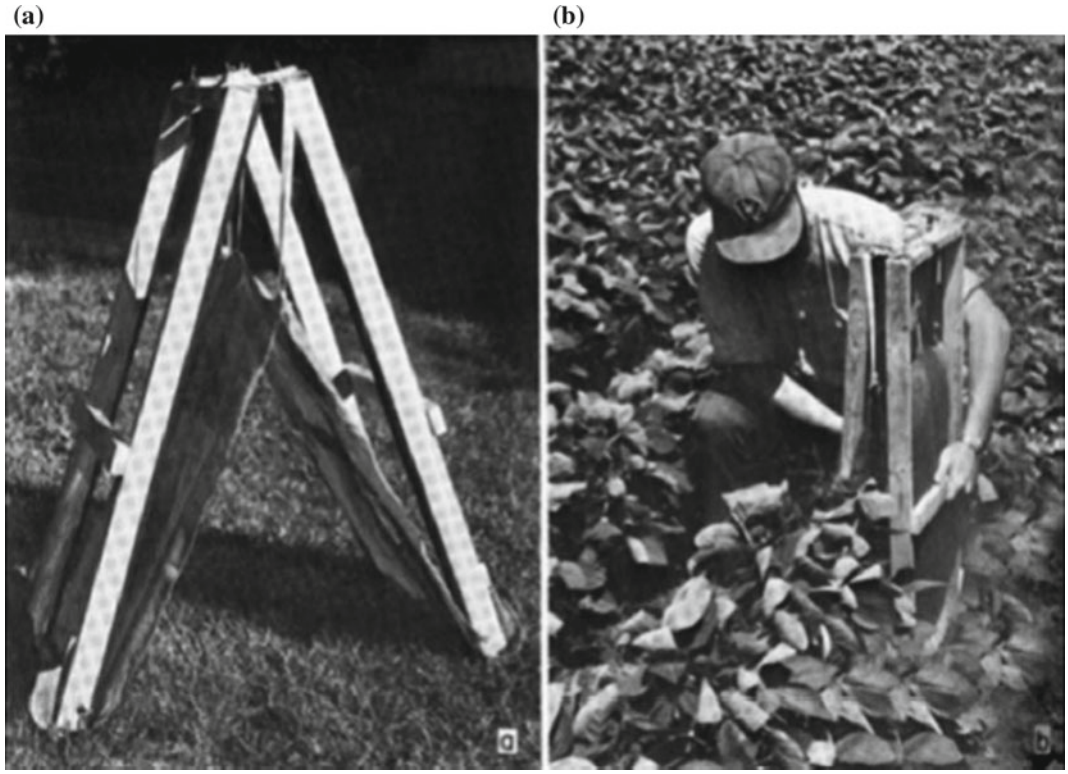

Fig. 6.8 **a** A clam trap used for sampling arthropods from a 30 cm segment of a row crop such as soybean; **b** use of clam trap in a soybean field. *Source* Mayse et al. (1978). Reproduced by permission of The Entomological Society of Canada

soapy water, and are retained in a sieve. Direct observation and clam trapping yielded similar results for the number of species recorded in different fields, but the results from sweep netting were much more variable, probably due to changes in vertical distribution of the arthropods sampled.

In summary, sweep nets, while frequently used as a tool for sampling arthropods in field crops, (and even, occasionally, in tree canopies, see Basset et al., 1997) are limited in their usefulness for investigations of natural enemy populations, and so should be used with caution, particularly if quantitative information is being sought. Also, like vacuum nets, they perform poorly under wet conditions. Further descriptions of sweeping techniques and the factors which influence sweep net catches are given by DeLong (1932), Saugstad et al. (1967), Kogan and Pitre (1980), Southwood and Henderson (2000).

6.2.4 Malaise Trapping

Malaise traps, first designed by René Malaise (Malaise, 1937), are tent-like interception devices that are particularly useful for obtaining large quantities of insect material for faunal surveys, studies of the relative abundance of species, phenological studies, studies of diurnal activity patterns, and taxonomic investigations (Gressitt & Gressitt, 1962; Townes, 1962; Butler, 1965; Matthews & Matthews, 1971, 1983; Ticehurst & Reardon, 1977; Steyskal, 1981; Sivasubramaniam et al., 1997). They are especially recommended for the collection of the adults of entomophagous insects such as Asilidae, Dolichopodidae, Empididae, Pipunculidae, Syrphidae, Tachinidae, and certain parasitoid Hymenoptera (Benton, 1975; Owen, 1981; Owen et al., 1981; Gilbert & Owen, 1990; Belshaw, 1993; Quinn et al., 1993; Hågvar et al., 1998).

Spider catches are usually small, but Malaise traps are useful for monitoring species that are not active on the ground and therefore are not caught in pitfall traps. Koomen (1998) caught 61 species of spider in one trap over two years. Traps are nowadays constructed of fine-mesh fabric netting, and incorporate a vertical panel (matt black in colour) that serves to direct insects upwards to the roof apex (there are a number of commercial suppliers of this type of trap e.g., B & S Entomological Services of Portadown, UK, and NHBS, of Totnes, UK) (Fig. 6.9). Alternatively, traps can be constructed from inexpensive lightweight materials, such as folding wood tripods secured with a bolt and wingnut and covered with spunbonded polyester netting. Platt et al. (1999) describe a 1.8 kg trap constructed from these materials that is easy to install and folds compactly for transport. Insects entering Malaise traps accumulate at the highest point within the roof (i.e., the apex) and pass eventually into a collecting bottle or jar that contains preservative (usually 70–95% ethanol or isopropyl alcohol or a sodium benzoate solution with detergent). 200 ml of 70% ethanol is sufficient for weekly collections in shaded habitats, but 250 ml are needed in habitats exposed to full sun (Cresswell, 1995). If diel activity cycles are under study, it is possible to replace the collecting bottle with a time-sorting mechanism (based on a quartz clock motor powered by a 1.5 V battery) which directs the 24 h catch into 12 Perspex tubes at 2 h intervals (Murchie et al., 2001). Individual Malaise traps can be highly variable in their catch rates (Longino & Colwell, 1997). For most kinds of insect, siting and orientation of traps is likely to be crucial; boundaries between different vegetation types, e.g., the edges of forest clearings, should be used, to exploit the fact that insect flight paths tend to be concentrated in such areas, while the collecting chamber end of the trap ought to point towards the Sun's zenith to exploit the positively phototactic responses of the insects. Malaise traps are normally positioned on the ground, but traps with rigid frames can be hoisted into tree canopy

Fig. 6.9 A Malaise trap of the type manufactured by Marris House Nets, UK

habitats (Faulds & Crabtree, 1995). Stork and Hammond (1997) have, however, shown that Malaise trapping in the canopy of an oak tree recovered half as many species of beetles as fogging (Sect. 6.2.5), and some of the most abundant species, known to be associated with oak, were absent. Unless diurnal activity patterns are being investigated, Malaise traps can be left for several days before emptying, although some workers report catches so large that daily collection is necessary (Gilbert & Owen, 1990). Malaise traps can be modified to record insects entering the trap from two opposite directions. Traps modified in this way were used by Hossain et al. (2002) to record parasitoids, ladybirds and hover flies dispersing from harvested into unharvested plots of lucerne.

Malaise traps do not provide data on the absolute abundance of insects, but they can yield useful biodiversity data. Gaston and Gauld (1993) reported on a network of Malaise traps operated at seventeen sites in Costa Rica for more than a hundred Malaise trap-years. One hundred and fifty species of Pimplinae (Ichneumonidae) were caught, and this was considered to be an accurate estimate of species richness, because: (a) when the catches of the seventeen sites were successively added together in a random sequence the curve for cumulative number of species plotted against cumulative number of sites approached an asymptote; (b) few extra species were added by casual collection using other methods at other sites in Costa Rica. This assessment of biodiversity was achieved in spite of Pimplinae being scarce in Costa Rica (overall mean of only six individuals caught per trap per month). Maes and Pollet (1997) considered Malaise traps to be useful for large-scale inventories of Dolichopodidae, but less suitable for ecological studies of these flies. This is because most dolichopodids fly for only short distances and a large number of traps would be necessary to counteract the resulting variability of catch.

An interception device similar in operation to the Malaise trap was devised by Masner and Goulet (1981). It incorporates a vertical polyester net treated with a synthetic pyrethroid insecticide. Intercepted insects crawling on the net are killed by the insecticide and fall onto a plastic tray placed beneath. A clear polythene roof minimises rain damage and deflects positively phototactic insects back onto the net. In the field, this design provided a larger catch of small-bodied Hymenoptera than did a Malaise trap (Masner & Goulet, 1981). Masner (in Noyes, 1989), and Campos et al. (2000), later improved the efficiency of the trap by setting a yellow trough into the ground below the intercepting vertical net. Noyes (1989) used a Masner-Goulet trap without treating the vertical net with insecticide, and obtained poor catches of parasitoid wasps compared with a Malaise trap, in tropical rain forest. Basset (1988) devised an apparatus that combined a Malaise trap with a window trap (Sect. 6.2.11) for sampling the canopy faunas of rainforest trees. Natural enemies either flew or crawled into the Malaise trap or collided with the Plexiglass sheet of the window trap and fell into the collection vessel (containing 20% ethylene glycol) below. Natural enemies reacted differently to the two trap components, which therefore varied between species in collection bias, and the results underscored the desirability of employing more than one sampling technique in community studies. Despite this, spiders were under-represented in the composite trap compared with canopy fogging (Sect. 6.2.5). Basset et al. (1997) describe other variants of composite flight-interception traps for use in tree canopies. When comparing Malaise traps and window traps (as separate pieces of apparatus) at a forest edge, Schneider and Duelli (1997) found that window traps were more effective for catching spiders, but Malaise traps were more efficient for Hymenoptera and Dermaptera. Finally, note that Malaise traps need to be located away from ants' nests, as the ants can severely reduce apparent catches by removing the caught insects.

Drift nets (the aquatic equivalent of the Malaise trap) can be anchored in streams at any required depth and used to sample aquatic insects moving downstream with the current. Daytime-only sampling could underestimate abundance, since much drift occurs at night (Williams & Feltmate, 1992).

6.2.5 Knock-Down

Knock-down involves the dislodgement of insects from their substratum (usually vegetation), causing them to fall onto either a tray, a funnel, a sheet or a series of such devices situated beneath.

Mechanical Knock-down

A common method employed in sampling invertebrates from vegetation is to either shake plants or beat them with a stick, causing the invertebrates to fall onto either a white cloth or polythene sheet laid on the ground, or onto a beating tray (a device resembling an inverted white umbrella, Fig. 6.10) (Jervis, 1979, 1980a). The fallen invertebrates are then either collected by hand or with an aspirator. Alternatively, they may be beaten into either a large plastic funnel fitted with a collecting jar containing a preservative (Basset et al., 1997; Rieux et al., 1999), a cloth funnel that directs them into a plastic zip-lock bag (Roltsch et al., 1998), or a semi-rigid polythene funnel which can be folded (Morris et al., 1999a).

Fig. 6.10 Use of a beating tray to collect insects from a tree canopy

This method is useful for estimating numbers of slow-moving arthropods which are easily dislodged from the plant, but it is advisable to calibrate the technique against other, absolute, sampling methods (Kogan & Pitre, 1980). The density of spiders knocked off soybean plants onto a ground cloth was not significantly different from the density estimated from plant fumigation (Culin & Rust, 1980). Majer et al. (1996) found that branchlet shaking was a viable alternative to chemical knock-down for sampling natural enemies such as spiders and small wasps (but not for tiny predatory mites). They reported that sixty 30 cm-long branchlets could be sampled per tree in 15 min and that four workers were able to sample ten trees adequately in a day. Fleischer et al. (1985) found that the beating method gave estimates of predator density in cotton that were less than one-third the size of those obtained by removing bagged plants to the laboratory. Beating has also been used to sample spiders from apple trees (Dondale et al., 1979) and to assess the numbers of adult anthocorid bugs, lacewings and ladybirds feeding on psyllids in pear trees (Hodgson & Mustafa, 1984). Beating and sweeping have been used to collect spiders from the forest canopy. This was facilitated by operating from a 'canopy raft' (i.e., a 580 m^2 inflatable hexagonal platform transported to the canopy by an air-inflated dirigible) (Basset et al., 1997). Beating is more commonly used than sweep netting to sample arthropods in trees, but Radwan and Lövei (1982) found that beating ladybirds from apple trees collected only 30% of the beetles observed by visual searching. Coddington et al. (1996) also found that beating was less productive than visual search for estimating spider species richness on hardwood trees. It is sobering that the samples were quite distinct taxonomically and that the two methods, although applied to the same vegetational strata, seemed to be accessing different components of the arachnofauna.

Pale-coloured insects may prove difficult to locate on white fabric such as that in a standard

beating tray. This problem may be overcome by using a dark-coloured fabric.

When beating is carried out in warm, sunny conditions, winged insects that fall on to the collecting sheet or tray surface tend to fly off from the latter very readily. It is therefore advisable in such circumstances to have a small team of workers who can remove insects as they land. An alternative is to use a beating tray modified to reduce the problem of escaping insects. Morris and Campos (1996) used a transparent polythene cone attached to a plastic ring which could be moved as easily as a sweep net. A detachable container was fitted below the cone to trap insects that were beaten into the apparatus. Newton and Yeargan (2002) determined the density of nocturnal harvestmen (Opiliones) in soybean by beating the plants at night (illuminated by headlamps) within a defined area temporarily enclosed by wooden boards. Dislodged harvestmen, and individuals secreted in the litter, were then collected from the ground.

Belshaw (1993) used a beating technique, together with a box trap (Fig. 6.11) to collect resting Tachinidae from ground vegetation. The box trap comprises a muslin-covered, open-bottomed metal frame. Using the handles, the device is placed quickly on the ground, and the vegetation thus enclosed is beaten with a stick. Winged insects (Tachinidae, in the case of Belshaw's study) disturbed in this way are collected either in the removable tube situated at the apex of the trap, or (via the sleeve, using an aspirator) from the insides of the cage. Because the box trap covers a known area of vegetation, Belshaw's technique can be used to obtain absolute measures of the density of insects. As the insects are not collected from beneath the plant, it could be argued that this is not, strictly speaking, a knock-down technique.

Comparison of beating sheets, sweeping and pitfalls in peanut fields showed that pests and natural enemies were represented to different degrees, on a species-by-species basis, using the three methods. It is recommended that at least two methods be used to assess the effect of a pest management practice on pests and beneficials (Kharboutli & Mack, 1993).

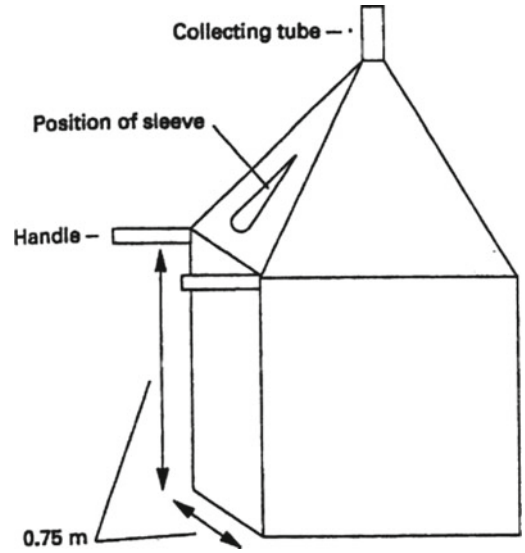

Fig. 6.11 Box trap used by Belshaw (1993) to collect insects, disturbed by beating, from ground vegetation. Reproduced by permission of the Royal Entomological Society of London

Chemical Knock-down

Natural enemies in tree canopies can be sampled directly and precisely from large expensive structures such as tower cranes (Parker et al., 1992) and canopy rafts (Basset et al., 1997) but, since most research budgets preclude the use of such equipment, chemical knock-down techniques provide a valuable alternative. Insecticide knock-down was pioneered by Roberts (1973) and is popularly known as fogging. It is being used increasingly in faunistic and other investigations of parasitoids and predators, particularly in forest canopy habitats and hedgerows (Neuenschwander & Michelakis, 1980; Noyes, 1984; Askew, 1985; Noyes, 1989; Joyce et al., 1997; Tobin, 1997; Watt et al., 1997; Stork et al., 2001; Veijalainen et al., 2013). A pyrethroid insecticide (e.g., Resilin E, Resilin 5OE; Noyes, 1989) fog is sprayed from the air, by aeroplane or helicopter, or is released from a device that is either held by the operator (Fig. 6.12a) or is hoisted into the tree canopy using a system of ropes and pulleys. Fitting a radio-controlled servo unit to the release valve of the fogger prevents wastage of insecticide and poor

targetting of fog as the machine is hoisted, or when its exhaust pipe is pointing in the wrong direction (Stork & Hammond, 1997). Adis et al. (1998) list the commercial fogging machines currently available. Insects can be collected by placing, beneath the canopy, either a sheet on the ground (this method may, however, lead to problems with ants plundering the catch) or several funnel-shaped trays slung from ropes (Fig. 6.12b) (Longino & Colwell, 1997; Stork & Hammond, 1997; Watt et al., 1997; Stork et al., 2001). Researchers have used a wide range of combinations of insecticides, delivery systems and arthropod collection methods, and Adis et al. (1998) make constructive recommendations for standardisation of protocols so that future data can be reliably compared. Catch size is not related to insecticide concentration in a simple manner and stronger concentrations do not necessarily increase the catch (Stork & Hammond, 1997).

After five minutes of fogging an Australian rainforest canopy with natural pyrethrins, Kitching et al. (1993) collected falling arthropods for 4 h and noted that 63% of the total catch was obtained by the first hour, 78% by the second and 90% by the third. Similarly, Stork and Hammond (1997) reported that 80% of the catch from birch

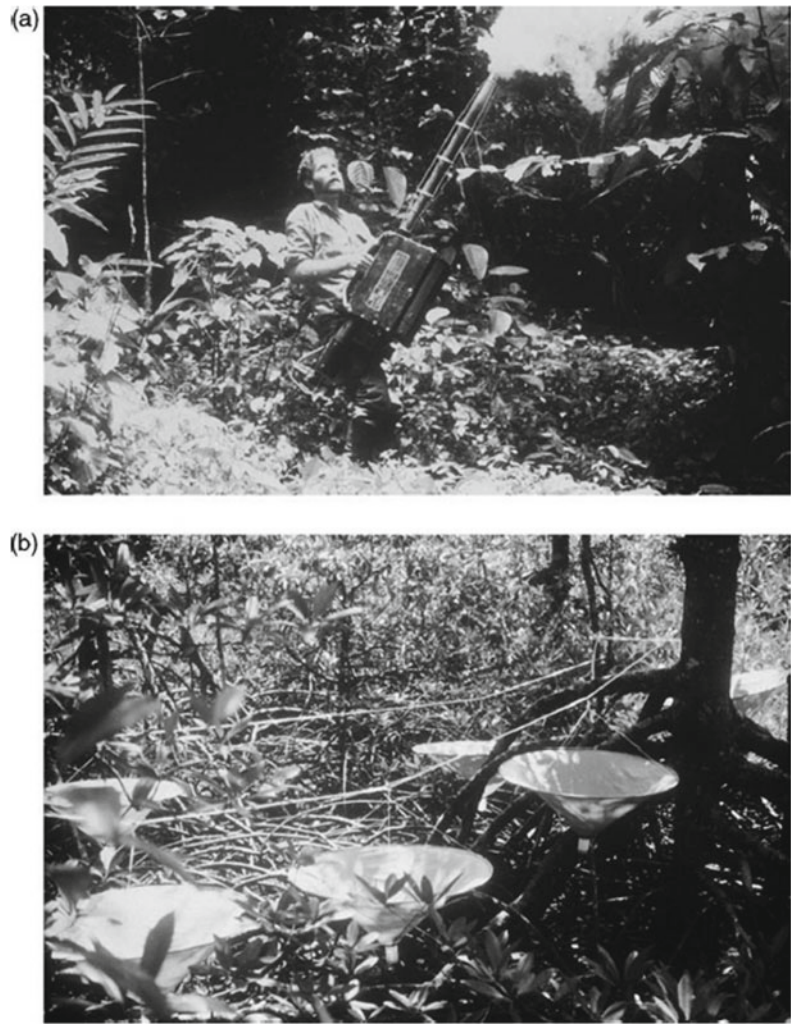

Fig. 6.12 Chemical knock-down of insects from the forest canopy, using the fogging technique: **a** application of the fog; **b** devices used to collect insects as they drop from the canopy. Reproduced by permission of Nigel Stork

trees in England were obtained within 75–85 min. It is not sensible to re-sample the same tree until recolonisation has occurred (Basset et al., 1997). Faunal recovery rates vary considerably due to factors that are incompletely understood, but may often exceed ten days (Stork & Hammond, 1997). Morning fogging collects fewer unwanted 'tourist' species than does evening fogging, at least in UK deciduous woodland (Stork & Hammond, 1997). Fogging of a whole tree canopy does not provide information relating to particular strata within the canopy, cannot be used effectively under windy conditions (when ground windspeeds are a gentle 5 km h^{-1} canopy windspeeds above 7 m can be in excess of 15 km h^{-1}), and loses any material that does not fall vertically (Majer et al., 1996; Basset et al., 1997). An approximate estimation of the stratum from which the natural enemies originate can, however, be obtained by concentrating the fogging in specific strata or by suspending the collecting funnels at various heights (Kitching et al., 1993). More precise distributional data can be obtained by 'restricted canopy fogging', which involves trapping the foliage in a 60,000 cm^3 conical plastic enclosure, exposing it to carbon dioxide at a pressure of 1 bar for twenty minutes, and then shaking the enclosure vigorously for a further ten minutes. Narcotised natural enemies are transferred to alcohol via a collector attached to the bottom of the enclosure. This method was found to extract 80–90% of arthropods from the foliage (Basset, 1990). A comparison of canopy fogging with restricted canopy fogging suggested that the latter collected more spiders and small invertebrates but fewer active flyers such as Diptera. Restricted canopy fogging, however, cannot be applied to wet foliage and does not collect invertebrates from the trunks of trees (Basset, 1990), and the foliage is disturbed when the enclosure is positioned (Basset et al., 1997). Stork and Hammond (1997) observed that some beetles and bugs will fly or drop off foliage as an escape reaction to even the minor disturbance of a sudden movement by the researcher, and these 'nervous' species are, therefore, likely to be underestimated by restricted canopy fogging. Floren and Linsenmair (1998) carried out tree-specific sampling of Coleoptera by fogging trees that had 100 m^2 cotton roofs fitted over their crowns, to exclude beetles from other trees of the higher canopy.

Based on experience with preparing an inventory of ant species in rain forest, Longino and Colwell (1997) noted that fogging is efficient in generating a large number of specimens in a short period of time, compared with Malaise traps, which must be left in the field for a long time to achieve the same result. For predators that are found across a broad range of habitats such as spiders, canopy fogging produces significantly different inventories from other trapping methods such as pitfall trapping, so for complete inventories more than one sampling method must be used (Pedley et al., 2016).

Mist-blowing is similar to fogging, but less dependent on wind speed and direction to transport the insecticide. Mist-blowing uses ultra-low-volume techniques to generate a fine mist which is propelled into the canopy (Chey et al., 1998). Use of a fluorescent marker showed that, with this technique, insecticide coverage of both leaf surfaces was similar and there was little variation between species of tree, but, when applied from the ground, the mist did not reach much higher than 7 m (Chey et al., 1998).

Chemical knock-down was used to sample parasitoids of Oriental fruit fly in guava orchards in Hawaii. Large plastic sheets were put under trees, a pyrethrin mist was blown into trees, which were then shaken vigorously for four minutes, and the parasitoids subsequently removed from the sheets. These parasitoids are poor flyers and unable to avoid the pyrethrin, which gives very fast knock-down (Stark et al., 1991).

Fogging can also be used for extracting insects from field crops; for example, Kogan and Pitre (1980) used a large, plastic fumigation cage in the collection of arthropods from soybeans.

6.2.6 Visual Counting

Although relative estimates of natural enemy densities are useful when investigating the effects

of experimental treatments, it is often desirable to obtain absolute density assessments for natural enemy populations in the field (Chaps. 1, 7 and 8). The most common way of doing this, especially for conspicuous life stages, is to visually count individuals *in situ*, on either a defined-area or period-of-search basis. This can extend to the visual searching of entire small trees to record absolute numbers of selected natural enemy species (Wyss, 1995). Coddington et al. (1996), in a study of spider species richness, compared visual search of the foliage of hardwood trees with beating, and visual search of the litter with litter extraction. Visual search yielded more species than other methods but there was a significant degree of variation in density and species richness attributable to the level of collecting experience of the four individuals conducting the survey. For the counting of less conspicuous stages or those in concealed locations, plants or other parts of the habitat can be removed to the laboratory for either extraction or examination (Southwood & Henderson, 2000) (Sect. 6.2.7). Visual counting is also a good method for examining spatial dispersion patterns of natural enemy species, provided that detectability of insects remains constant across the study area.

Lapchin et al. (1987) compared three methods of estimating densities of hover flies and ladybird beetles in winter wheat crops:

1. An observer walked at a steady pace through the crop and counted all adults and larvae seen within a period of 2 min (census-walk method).
2. An observer made a detailed search of plants and soil surface in a defined area, and, immediately afterwards, a second observer searched the same area. Predator density was then calculated using De Lury's method (Laurent & Lamarque, 1974): $P = C_1^2 / (C_1 - C_2)$, where P is predator population density, C_1 is the number of predators collected by the first observer and C_2 the number collected by the second. The method assumes equal capture efficiency for the two counts, and no emigration or immigration between counts.
3. Plants were cut and removed to the laboratory for detailed examination.

The visual searches carried out in the field (methods 1 and 2) proved to be unsuitable for estimating the densities of hover fly larvae because of the insects' cryptic colouration, their relative immobility and their habit of resting in concealed positions on plants. The population density of hover fly larvae estimated by detailed searches in the field was less than 1% of that estimated from plant removal and examination (Sect. 6.2.7). The detailed searches using De Lury's method were, however, adequate for assessing absolute numbers of ladybird adults and of final-instar larvae, although they were not reliable for earlier instars. The census walks provided less accurate estimates of absolute density but they were satisfactory for determining seasonal trends in predator numbers, especially numbers of adult ladybirds.

Chambers and Adams (1986) also used detailed visual searches to estimate numbers of hover fly and of ladybird eggs and larvae in winter wheat. Plant shoots were searched in situ and additionally the shoots in 0.1 m^2 quadrats were cut and counted and the resulting stubble, together with the soil surface and any weeds present, was searched. Mowes et al. (1997) showed that counting predators on tillers of winter wheat was nearly as accurate as 'absolute' density sampling (suction sampling in a cage and examination of cut vegetation in the laboratory) for Coccinellidae, Syrphidae and Chrysopidae, but not for the majority of groups of polyphagous predators. Detailed visual searching is labour-intensive, but Poehling (1987) concluded that, although it was the most accurate method of estimating ladybird and hover fly numbers in cereal crops, it was not appropriate when large numbers of experimental plots needed to be assessed in a short time. It is especially labour-intensive in habitats with dense vegetation. Duffey (1974) found that 60–90 person-minutes were needed to search 0.5 m^2 of calcareous grassland and remove the resident spiders. Mobile predators on exposed surfaces (such as

tree trunks and branches) are more evident and counting is easier. Peng et al. (1999) estimated abundance of the green ant, *Oecophylla smaragdina*, on cashew by counting the number of ant trails on the main branches and by ranking trails according to the amount of ant activity present.

Frazer and Gilbert (1976) used a census-walk method to assess adult ladybird densities in alfalfa fields. This entailed walking along either side of each crop row and counting all beetles that were visible. These counts were, however, influenced by the weather (beetles being more active and therefore more easily seen when temperatures were high), so they also searched 30 cm lengths of crop row more thoroughly. Even so, they found that these counts never exceeded 25% of the beetles actually present, because the ladybirds spend most of their time in the stubble at the base of the crop. This finding highlights the influence of insect activity on the efficiency of visual searches and census walks in the field, active individuals being far easier to see than inactive ones. Both the degree of predator hunger and the time of day can affect the activity of ladybirds (Frazer & Gill, 1981; Davis & Kirkland, 1982). Time of day can also affect counts of hover fly larvae; twice as many were found during detailed night-time searches compared with daytime searches of plants and the soil surface in an oat crop (Helenius, 1990). A further factor influencing visual field assessments is the observer's experience with the method. When assessing adult ladybirds in strawberry crops using census walks, Frazer and Raworth (1985) noted significant differences between the numbers recorded by different observers. Similarly, variation among observers accounted for a large proportion of the error in predator density estimates from visual searches in cotton crops (Fleischer et al., 1985). Because of the effect of hunger on activity, Frazer (1988) advocates the use of sampling procedures which are specifically designed to estimate only the numbers of active, hungry individuals where predictions of future predation rates are required. Weseloh (1993) used a census-walk method to determine the abundance of ants in different plots.

It is possible to obtain data on the densities of adult parasitoids using visual counting in the field, if the parasitoids are either large-bodied and conspicuous or slow-moving. Many parasitoids search the food plants of their hosts by walking, so facilitating visual observations in the field. The numbers of adult *Diadegma* ichneumonid wasps foraging over Brussels sprout plants in an experimental plot were recorded by a pair of observers using binoculars (Waage, 1983). The observers checked all sides of each plant but did not approach closer than 5 m, to avoid disturbing the wasps. Hopper et al. (1991) counted adult *Microplitis croceipes* (Braconidae) along 60 m rows of cotton plants, catching each individual with a hand net in order to establish its sex.

Obviously, census-walk methods are only appropriate for highly conspicuous insects, and the methodology that has been developed for monitoring butterfly populations in Britain (Pollard, 1977) could be applied to certain natural enemies, such as ladybirds, hover flies, dragonflies or large-bodied parasitoid species. Because weather affects flight activity, butterfly census walks are not useful at temperatures below 13 °C, and between 13 °C and 17 °C they are only carried out in sunny conditions (Pollard, 1977). It is also important to define the distance limits, on either side of the observer, within which the counts are made during a census walk. During the butterfly counts, use is made of natural features such as footpaths or forest rides, but for census walks through crops, boundaries can be defined in terms of crop rows or by placing markers, such as bamboo canes, along the route. The butterfly census-walk data are used to calculate indices of abundance for individual species at each census site. The mean count per census walk is calculated for each week and then summed across weeks to give the index of abundance for the season. Population changes from year to year can then be assessed by comparing these indices.

In some cases, parasitised insects can be readily distinguished from healthy individuals, either in the later stages of parasitism, or immediately after death, and their numbers assessed by

visual counting. An obvious example is the 'mummification' of parasitised aphids. Aphid 'mummies', resulting from parasitism by aphidiine braconids (Fig. 6.13) or Aphelinidae, act as protective cells within which larval parasitoids complete their metamorphosis to the adult stage. Mummies are conspicuous within aphid colonies and are therefore readily counted in situ. Consequently, mummy counts are frequently used to estimate aphid parasitoid abundance (Lowe, 1968; van den Bosch et al., 1979; Carter & Dixon, 1981; Messing & Aliniazee., 1989; Feng & Nowierski, 1992; Hance, 1995; Lapchin et al., 1997). It is, however, advisable to combine this sampling technique with other methods of assessing parasitoid populations, such as the rearing or dissection of parasitoids from samples of live aphids (Sects. 6.2.9, 6.3.5, 6.3.6), since mummy counts alone can give misleading results for several reasons:

Fig. 6.13 Aphid 'mummies': *Sitobion avenae* parasitised by *Aphidius rhopalosiphi* (Braconidae) on ears of wheat

1. Some parasitised aphids leave their colonies, and may even leave the food plant altogether, prior to mummification (Powell, 1980; Dean et al., 1981; Brodeur & McNeil, 1992).
2. Many aphid parasitoids pass through diapause in the aphid mummy and the proportion doing so increases as the season progresses (Singh & Sinha, 1980), leading to the accumulation of mummies in the study site, and so increasing the likelihood that the same individuals are counted in successive samples.
3. Heavy rain or strong wind may dislodge mummies from plant leaves.

Visual searching of plants in the field or of cut plants in the laboratory has been used to assess predators on a variety of other field crops including soybeans (Kogan & Herzog, 1980), sorghum (Kirby & Ehler, 1977), brassicas (Smith, 1976; Horn, 1981; Landis & van der Werf, 1997) and cotton (Fleischer et al., 1985). Fleischer et al. (1985) placed cylindrical cloth bags over whole cotton plants, tying the bottom securely around the base of the main stem. The bags were left collapsed on the ground for a week and then two people rapidly pulled the bags up over the entire plants, tying off the top before removing bagged plants to the laboratory for subsequent visual examination.

Soil surface-dwelling polyphagous predators are more difficult to assess visually in the field because many of them are active mainly at night, and spend much of the daytime concealed in the litter on the soil surface, under stones or in the soil itself. Brust et al., (1986a, b) estimated ground predator numbers in corn by placing metal quadrats (13 × 75 cm) over plant rows and visually searching the surface litter and the soil to a depth of 0.5–1.0 cm. Winder et al. (1994) searched within quadrats, then used a trowel to transfer weeds, stones and plant roots to a plastic tray for further examination.

Sunderland et al., (1986a, 1987a) estimated linyphiid spider densities in cereal fields by searching the crop and the top 3 cm of soil within 0.1 m^2 quadrats, and collecting the spiders with an aspirator. Nyffeler (1982) used 1 m^2 quadrats for counting large spiders on vegetation, but

0.04–0.16 m^2 quadrats for counting small spiders on the soil surface. Harwood et al., (2001a, 2003, 2004) used 0.008 m^2 mini-quadrats, which had sufficient spatial precision to enable predators and prey numbers to be quantified both within web sites of linyphiid spiders and separately in areas between web sites. At this scale it is possible to analyse invertebrate densities within confined microhabitats, such as within and between rows of a cereal crop. Use of quadrats may underestimate the density of large, active adult spiders (such as Lycosidae) which can flee from the area of disturbance. Nyffeler and Benz (1988a) used a different method to estimate numbers of linyphiid spiders in winter wheat fields and hay meadows; they counted the number of webs within randomly placed quadrats, but conceded that their counts were probably underestimates, because some spiders occupied cracks in the soil. Toft et al. (1995) used potato starch powder to reveal webs, then measured the distances from a random point in the field to the ten nearest webs. 'Distance methods' (Krebs, 1999; Southwood & Henderson, 2000) were then used to estimate the density of web-building spiders; e.g., $V_1 = n/\pi \sum \left(X_i^2\right)$, where N_1 = estimate of population density from point-to-web data, n = sample size, and X_i = distance from random point to nearest web. Key deficiencies of this method are that not all individual spiders produce webs, and some species produce webs on a daily basis, these may remain intact for several days or be destroyed immediately by heavy rain.

Sunderland et al. (1987b) attempted to achieve an accurate estimate of total predator density in cereal crops by combining a range of sampling techniques. Insects were first extracted using a vacuum net with a sampling head which covered 0.1 m^2. The sampled area was then isolated by means of a metal cylinder which was driven into the ground to a depth of 8–10 cm, and the plants within this area were cut and removed to the laboratory for close visual examination. The soil surface was searched visually and the predators were collected with an aspirator. Next, pitfall traps were set in the enclosed area which was further isolated by sealing the top of the metal cylinder with a fine-mesh net. It was concluded that any single sampling method will underestimate predator density, and that a more accurate estimate is obtained by combining different methods. In any system, the advantages of intensive sampling methods must be balanced against the disadvantages of excessive labour requirements. Increased effort at each sample site will be traded off against the number of replicate sites that can be managed, potentially reducing the statistical power of an ecological study.

6.2.7 Extraction of Natural Enemies from Living Plant Tissues, Leaf Litter and Soil (Including Emergence Traps and Soil-Flooding)

Some natural enemies spend part of their life cycle concealed, along with their prey or hosts, within plants. The prey- or host-infested parts of the plants can be removed for subsequent dissection. In some cases, the plants infested by the hosts or prey, e.g., gall-causing insects and leafminers, are relatively easy to distinguish from uninfested ones, and these alone need to be collected. Plants containing the early stages of gall-causers, and those containing borers, can be difficult or impossible to recognise. The removal of whole plants or parts of plants for subsequent close examination is the most efficient way of counting life stages that are not highly mobile. Harris (1973) assessed several methods for estimating numbers of aphidophagous cecidomyiid fly larvae attacking aphid colonies on a variety of plants. Visual searches in the field proved difficult when aphid colonies were very dense or occurred in protected situations such as curled leaves or galls. An alternative method was to place samples of aphid-infested plant material into polythene bags and keep these in the laboratory for 2–3 days. Subsequently, older predator larvae left the aphid colonies and could be counted as they crawled on the inner surface of

the bag. The most efficient method was to shake samples with 70% ethyl alcohol in polythene bags and then wash them into a plastic dish where insects could be brushed from the plant material with a soft brush. After removal of larger individuals, the samples were washed through a series of filters to retrieve the eggs and small larvae. Field searches and incubation in polythene bags were respectively only 11% and 32% as efficient as the alcohol-washing method.

Collection of plant parts can be extended even to sampling from large trees. Abbott et al. (1992) used a cherrypicker to cut branchlets from the crowns of jarrah (*Eucalyptus marginata*) at 14 m above ground. Each branchlet was allowed to fall into an open plastic bag held just below the branchlet, invertebrates were killed immediately by adding to the bag a wad of cotton wool soaked in ethyl acetate, and the contents of the bag were later sorted and identified in the laboratory. Pettersson (1996) sawed branches off spruce trees, transported them wrapped in plastic, and then collected spiders by hand as the branches dried out in the laboratory. Similarly, Halaj et al. (1998) harvested and bagged 1 m-long tree branches in a survey of spider abundance and diversity in forest canopies. Sampling for parasitoids of wood-boring beetles can involve felling large trees, cutting branches above a certain diameter into sections, and dissecting bark with chisels and hammers to extract host larvae for parasitoid rearing (e.g., Liu et al., 2004).

Predators or parasitoids concealed in soil or leaf litter can be studied by taking a sample of the concealing medium and extracting the natural enemies either by hand (Dubrovskaya, 1970; Franke et al., 1988; Thomas et al., 1992), by extraction equipment (Duffey, 1962; Kempson et al., 1963; Edwards & Fletcher, 1971; Bonkowska & Ryszkowski, 1975; Pollet & Desender, 1985; Hassall et al., 1988; Helenius et al., 1995; Pfiffner & Luka, 2000) such as a Tullgren funnel apparatus (Workman, 1978; Chiverton, 1989), or by soil washing and flotation (Desender et al., 1981; Dennison & Hodkinson, 1984; Sotherton, 1984, 1985), or by various combinations of these methods (Alderweireldt, 1987; De Keer & Maelfait, 1987, 1988). If the aim is to quantify species composition, it should be noted that the efficiency of heat extraction techniques, such as Tullgren funnels, can vary greatly between taxa of invertebrates, and even between species within a family (Snider & Snider, 1997). The Winkler/Mozarski eclector is a suitable litter extractor for expeditions because it does not require a source of power and is lightweight (five eclectors can be transported easily in a rucksack). This extractor exploits the escape responses of disturbed invertebrates which pass through the nylon mesh of a litter bag into a collecting vessel placed below (Besuchet et al., 1987). Edwards (1991) reviewed many of these extraction techniques and concluded that dry funnel techniques are most efficient for micro- and mesoarthropods, but flotation techniques are more efficient for macroarthropods. To sample ants in forest and grassland habitats, Morrison (2002) searched under rocks, logs and litter, then extracted ants from the leaf litter using Berlese funnels and the Winkler method. Sotherton (1984) used a spade to dig out soil cores (20 cm $\times$ 20 cm), to a depth of 35 cm or to bedrock, which were extracted by breaking them up into a large container of saturated salt solution. Organic matter which floated was removed using a fine-mesh sieve. Predators were then extracted by hand. Spence and Niemelä (1994) used a similar method to collect carabids from litter. Litter from 0.25 m^2 of forest floor was placed in a large container of water and carabids were collected as they came to the surface. This litter washing technique was 96% efficient (based on recovery of marked beetles added to 'litter samples'), and estimates of carabid density for litter washing were twice those for Tullgren funnel extraction. The efficiency of extraction can be determined by adding known numbers of natural enemies to a unit of defaunated habitat, or, alternatively, by carefully searching the processed habitat sample for unextracted corpses. The first method is expected to understimate and the second to overestimate true efficiency (Sunderland et al., 1995a) and so the correct efficiency value is likely to fall between the two estimates.

Removal of samples of habitat from aquatic systems can be achieved using a freeze-corer, Ekman grab, or multi-core sampler (see details in Williams & Feltmate, 1992). Over the years, various devices have been designed for extracting samples of vegetation from aquatic habitats (Hess, 1941; Gerking, 1957; James & Nicholls, 1961; McCauley, 1975; Williams & Feltmate, 1992). An extensive review of these and other techniques for sampling aquatic habitats can be found in Southwood and Henderson (2000). The substratum from areas of stream beds, delineated by quadrats, can be removed with the aid of a Surber sampler (Surber, 1936) and taken to the laboratory for extraction of invertebrates, including predators (Hildrew & Townsend, 1976, 1982; Woodward & Hildrew, 2002). Since the Surber sampler does not catch all the invertebrates disturbed from the stream bed, it cannot be used for absolute population estimates (Southwood & Henderson, 2000).

Adult insects emerging from soil (e.g., from pupae contained therein) can be collected using an emergence trap (Southwood and Henderson (2000) describe various designs). Such traps comprise a metal, plastic or opaque cloth box that covers a known area of the soil surface. Insects are collected in transparent vials situated at either the sides or the top of the box. Emergence traps can also be constructed from wooden or perspex frames (e.g., enclosing 0.5–1 m^2), covered with fine mesh, and containing two to four pitfall traps emptied weekly or fortnightly (Helenius et al., 1995; Purvis & Fadl, 1996; Holland & Reynolds, 2003). When the emergence trap is in the form of a large pyramidal tent, with a collecting chamber at the apex, it is sometimes termed a photoeclector. Photoeclectors have been used extensively for monitoring and collecting natural enemies in crops (Funke, 1971; Törmälä, 1982; Bosch, 1990; Büchs, 1991, 1993; Wehling & Heimbach, 1991; Kleinhenz & Büchs, 1993). A pitfall trap can be used inside the photoeclector (Mühlenberg, 1993) to remove ground predators and prevent predation on emerging natural enemies, but, to be effective, it should be positioned near the wall of the photoeclector (Kromp et al., 1995). If emergence traps are to be used in studying phenologies, it should be borne in mind that the construction of the trap will influence the microclimate above the enclosed area of soil. All traps tend to reduce daily temperature fluctuations (the deeper the trap, the smaller the fluctuations), and the development rate of pupae may be affected (Southwood & Henderson, 2000). Emergence traps have been used to study ground predators such as carabid beetles (Helenius & Tolonen, 1994; Helenius, 1995), spiders (Duffey, 1978), predatory Diptera (Delettre et al., 1998) and parasitoid wasps (Jensen, 1997; Moore, 2001). If traps are emptied frequently at regular intervals, temporal emergence patterns can be recorded. There are few data on trap efficiency; an exception is the study of Moore (2001) which showed that 80 of 100 adult *Bracon hylobii* (a braconid parasitoid of the large pine weevil, *Hylobius abietis*) released into an emergence trap were captured within 6 h.

Arboreal emergence traps (Basset et al., 1997) can be used to investigate predators living in or on the bark of trees. Reeve et al. (1996) used such traps to monitor *Thanasimus dubius*, a clerid beetle predator of the southern pine beetle (*Dendroctonus frontalis*). One-metre vertical sections of pine trunk were enclosed with polyethylene screening leading down to funnels constructed of galvanised metal screening, which terminated in collecting jars filled with ethylene glycol solution. This method was used for two years and demonstrated a number of distinct emergence periods for *T. dubius*. Time-sorting arboreal photoeclectors attached to the trunks of oak and pine trees demonstrated a range of diel activity patterns in the spider community of this habitat. The photoeclector was a funnel of black cloth attached to a plastic tube, with a sampling jar mounted above. An electric motor, powered by two car batteries, was used to drive a turntable that changed the sampling jar at 6 h intervals (Simon et al., 2001).

Aquatic insects, emerging as the adult flying stage, move upwards through the water from the substrate and can be caught in a Mundie pyramid trap or a Hamilton box trap. These are essentially cages placed on the substrate that direct emerging insects into collecting jars at the apex of the

trap. Alternatively, simpler and cheaper floating emergence traps (e.g., cups suspended from styrofoam board) can be used to catch the insects when they reach the surface (Williams & Feltmate, 1992).

Soil-flooding techniques can be used to extract predators from soil in situ (Desender & Segers, 1985; Brenøe, 1987; Basedow et al., 1988; Kromp et al., 1995). Isolators are sunk into the ground, the vegetation examined and removed, then 2 L of water are added to each 0.1 m^2 isolator. Predators that emerge are removed and then another 2 L of water are added and any late-emerging predators are also removed. The process is reasonably rapid (taking about 10 min per 0.1 m^2 isolator), but large volumes of water must be transported to the site and the method is ineffective under some soil conditions (Sunderland et al., 1995a). Gradual flooding of soil samples taken back to the laboratory, a standard technique for measuring slug biomass in the field (Glen et al., 1989), has also proved useful for extracting carabid larvae (Thomas, 2002).

Efficient extraction of tiny predators, such as phytoseiid mites, from plant substrates requires a degree of inventiveness. To remove phytoseiid mites from apple leaves the sample of leaves was soaked in water and detergent for 2 h then leaves were washed on both sides and the washings directed through a filtering apparatus to be caught eventually on a small circular net, which could be transferred directly to a stereomicroscope. Examination of processed leaves showed that all mites had been successfully removed by this procedure (Jedlicková, 1997). As an alternative to water and detergent, an aqueous solution containing ethyl alcohol, sodium hypochlorite and liquid detergent was found to be effective for removing phytoseiid mites from lime leaves (Childers & Abou-Setta, 1999). Overwintering phytoseiids were removed from sections of wood from Australian grapevines using a microwave oven. Sections of wood in plastic bags were given 6 min at 120W and this brought the mites out of their hiding places in fissures in the wood. The method is expected to be more efficient than Berlese funnel extraction and also dislodges Tenuipalpidae, Bdellidae, Anystidae, Parasitidae and Tydeidae (James et al., 1992). Mites can be dislodged from leaves using a brushing machine (Chant & Muir, 1955). Leaves are passed between a pair of contrarotating cylindrical brushes and dislodged mites fall onto a rotating sticky disc. This method is quick, efficient and allows accurate identification of species and developmental stage. If the samples are examined immediately after brushing, living mites can be distinguished from dead ones. Mite-brushing machines are commercially available from several entomological equipment suppliers.

6.2.8 Sampling by Attraction

It is possible to exploit the attraction responses shown by natural enemies towards certain stimuli (Chap. 1) in order to develop sampling techniques that can provide information on phenology and relative abundance. Data collected by attraction for comparative purposes, however, need to be treated with caution because different species will vary in their level of response to the same stimulus. Obviously, absolute estimates of insect abundance cannot be obtained using these methods.

Visual Attraction

Many winged insects are attracted to certain colours during flight, most commonly to yellow. Consequently, shallow, coloured trays or bowls filled with water, in which the attracted insects drown, are frequently used as traps to sample flying insects (Southwood & Henderson, 2000) (Fig. 6.14a, b). A preservative, such as 10% formalin or 1% formaldehyde, or sodium benzoate (Wratten et al., 2003), can be used in the water traps, and it is advisable to add a few drops of detergent if the target natural enemies are small and otherwise able to walk on the surface film, as was found to be the case for small dolichopodid flies (Pollet & Grootaert, 1996). Principally designed to attract phytophagous insects, yellow-coloured water traps also catch some groups of natural enemies, with varying levels of efficiency. They have been used in cereal crops where adult hover flies were the

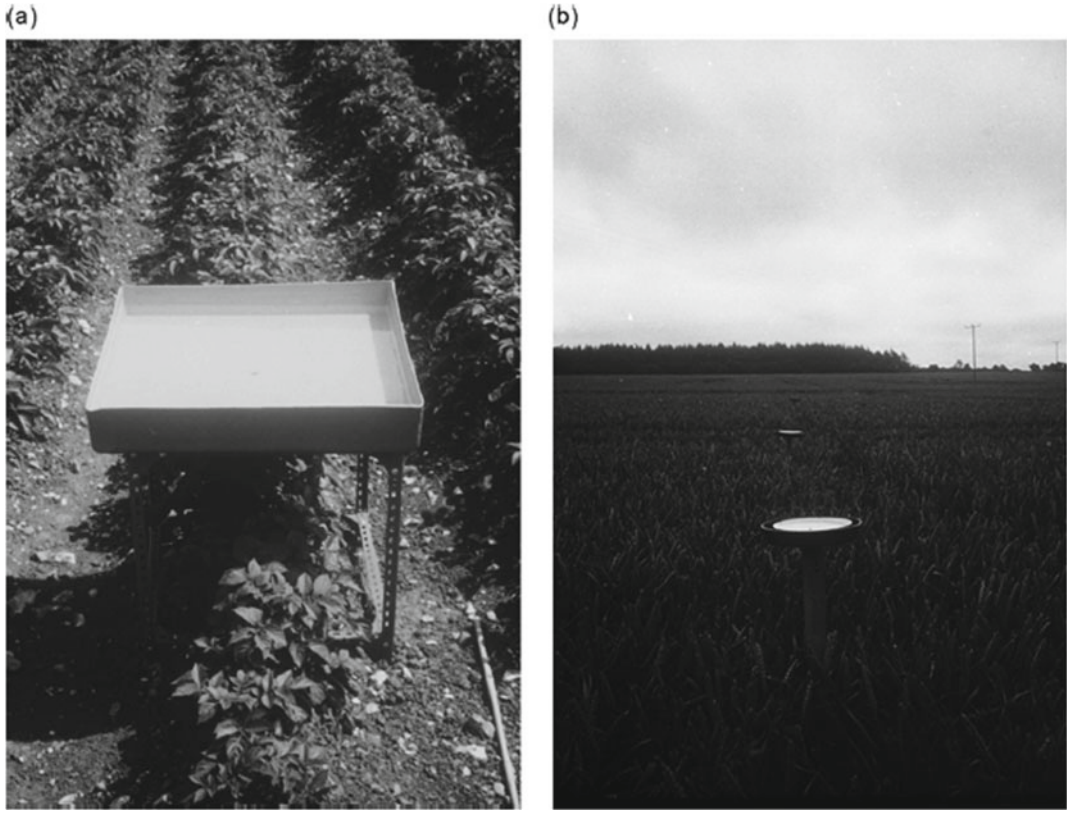

Fig. 6.14 Yellow water traps: **a** trap used by entomologists at Rothamsted to sample insects such as adult hover flies and parasitoids in a potato crop; **b** traps arranged in an experimental cereal field (arranged perpendicularly to the field edge), used by entomologists at the University of Southampton to measure the within-field distribution and abundance of adult hover flies

most abundant predators caught, although adult lacewings and adult parasitoids also occurred in the traps (Storck-Weyhermüller, 1988; Helenius, 1990). The relative abundance of adult hover flies, anthocorid bugs and parasitoids has been assessed in Brussels sprout fields using yellow water traps (Smith, 1976). Yellow (Delettre et al., 1998) and white (Pollet & Grootaert, 1987, 1996) water traps have been used to monitor predatory Empidoidea (i.e., dolichopodid and empidid flies) in natural habitats. Pollet and Grootaert (1993) reported that yellow water traps caught the largest number of individuals of Empidoidea, but white ones provided a more reliable estimate of species diversity in woodland. Such generalisations obscure the variation that can occur between species. For example, Pollet and Grootaert (1987) caught 60 species of Dolichopodidae in coloured water traps in a humid woodland, and, although the majority of species were caught in white or red traps, *Sciapus platypterus* showed a marked preference for blue traps. Blue-green traps raised 60 cm above the soil surface were found to be the best option for catching arboreal Empidoidea (Pollet & Grootaert, 1994).

The attractiveness of yellow water traps to individual insects will depend to some extent on the latters' physiological condition. Adult hover flies, for example, are caught more readily when they are newly emerged and/or hungry, and when food sources are scarce (Schneider, 1969; Hickman et al., 2001). Similarly, females of the parasitoid *Cotesiarubecula* are more likely to search out yellow targets when starved, than when fed on sugar solution (Wäckers, 1994). Traps of various colours have been used to catch adult

hover flies (Dixon, 1959; Sol, 1961, 1966). Water traps of 32 different colours were tested in a UK cauliflower crop. The colour 'fluorescent yellow' caught significantly more hover flies than other colours (Finch, 1992), and it was also demonstrated in the laboratory that the hover fly *Episyrphus balteatus* is attracted to yellow more than white, green or blue (Sutherland et al., 1999). Schneider (1969) suggested that colour preference may be influenced by the colour of the most abundant flowers in bloom at the time of sampling. Sol (1966) defined the colours used in his water trap studies on the basis of human interpretations of colour, but a better approach, adopted by other workers (such as Kirk, 1984; White et al., 1995; Wratten et al., 1995), is to define colours in terms of ultraviolet reflectance spectra. Even apparently colourless surfaces (e.g., translucent plastic) may have UV reflectance properties (M. A. Jervis, personal communication). Kirk (1984) recorded two species of predatory fly of the genus *Medetera* (Dolichopodidae) in water traps of seven different colours, and found most in white and blue traps, with very few in yellow traps. Although the rank order of the catches for each colour was similar in both species, the proportions caught by each colour of trap were different. Obviously, great care must be taken when estimating the relative abundances of different species from coloured trap catches. Wratten et al. (1995) compared the attractiveness of water traps of different colour to two species of hover fly in New Zealand. *Melangyna novaezelandiae* preferred yellow (also later confirmed by Bowie et al., 1999 for *Melangyna viridiceps* in Australia), but *Melanostoma fasciatum* showed no significant preference between yellow, white and blue. Trap reflectance was measured with a UV–visible spectrocolorimeter. Daily trap emptying gave 15% larger catches than infrequent emptying. This may have been because the accumulation of dust, leaves and insects in the infrequently emptied traps reduced trap reflectance.

The attraction of hover flies to coloured water traps is based on their visual attraction to flowers as pollen and nectar sources. Many adult parasitoids also feed on nectar and pollen (Chap. 8) and are therefore likely to respond to coloured traps when foraging for such resources, although it has been suggested by Helenius (1990) that some parasitoids, such as those attacking aphids, may lack a behavioural response to yellow traps, because they feed on host hemolymph or honeydew (but see Jervis et al., 1993). Visual cues, such as certain colours, may very well be used in host habitat location by adult parasitoids. In choice tests, two egg parasitoids of stink bugs (*Telenomus podisi* and *Trissolcus basalis*) displayed strong innate responses to yellow substrates over green, black, brown, or white substrates (Aquino et al., 2012). This response did not change when parasitoids were trained to associate hosts with innately less attractive substrates. Goff and Nault (1984) tested the pea aphid parasitoid, *Aphidius ervi*, for its response to transmitted light and recorded the strongest response to green (wavelength 514 nm). Ogino et al. (2016) demonstrated that recruitment of the the predatory bug *Orius sauteri* can be increased with violet light (405 nm), and that this recruitment results in increased prey (thrips) suppression. When used in field crops, water traps are usually positioned level with, or just above, the top of the crop canopy, but they have also been used successfully when placed near the ground between the trees in orchards, forest plantations or natural forest habitats (Noyes, 1989). Arthropod activity, and hence size of catch in water traps, can vary with height above ground. Vega et al. (1990) describe an adjustable water trap that can be positioned at any height up to 2.2 m. Traps of an unspecified colour were used to sample adult anthocorid bugs, which attack psyllids in pear orchards (Hodgson & Mustafa, 1984). For some of the species caught, there was a significant relationship between the numbers of anthocorids in the water traps and the numbers present on the trees, as estimated by beating. Yellow pan traps are particularly efficient in the sampling of parasitoid wasps, such as Ceraphronidae, Scelionidae, Platygastridae, Diapriidae, Mymaridae and Encyrtidae (Masner, 1976; Noyes, 1989). Stephens et al. (1998) found that more parasitoid wasps were caught in yellow pan traps than in white ones.

The attractiveness of certain colours to insects has also been employed in the sampling of predators and parasitoids with sticky traps (Wilkinson et al., 1980; Weseloh, 1981, 1986b; Neuenschwander, 1982; Trimble & Brach, 1985; Ricci, 1986; Samways, 1986; Bruck & Lewis, 1998; Heng-Moss et al., 1998; Hoelmer et al., 1998; Larsen et al., 2014; Böckmann & Meyhöfer, 2017). Trimble and Brach (1985) examined the effect of colour on sticky trap catches of *Pholetesor ornigis*, a braconid parasitoid of leafminers. Yellow (of various shades) and orange traps were significantly more attractive than red, white, blue or black traps, and spectral composition appeared to be more important than reflectance. Yellow was also more attractive than other colours to *Macrocentrus grandii*, a braconid parasitoid of the European corn borer, *Ostrinia nubilalis* (Udayagiri et al., 1997). In contrast, high reflectance, regardless of colour, seemed to be important in the attraction of tachinid parasitoids to sticky traps placed in forests (Weseloh, 1981). The aphelinid parasitoid, *Aphytis melinus*, was caught in greater numbers on green and yellow sticky cards than on white, blue, fluorescent yellow, black, red or clear cards (Moreno et al., 1984), whilst eight times as many *Aphidius ervi* (Braconidae Aphidiinae) were attracted to green sticky cards than to gold, blue, white, red, black or orange cards (Goff & Nault, 1984). Coloured balls of various sizes (to simulate fruit) were coated with sticky material and hung from guava trees in Hawaii. Some species of parasitoid of the Oriental fruitfly, *Bactrocera* (= *Dacus*) *dorsalis*, were attracted to balls of specific colours (Vargas et al., 1991). The opiine parasitoids of this pest were more attracted to yellow than to darker colours, and capture rates on red spheres were very low (Cornelius et al., 1999). Coloured papers were inserted into glass tubes coated with sticky material (Tanglefoot®) to investigate the colour preference of naturally occurring *Trichogramma* in sorghum, as part of an investigation of how these parasitoids locate their host habitat (Romeis et al., 1998). Coloured sticky traps can also be used to study diel variation in parasitoid flight activity. Idris and Grafius (1998) showed that the number of *Diadegma insulare* (a parasitoid of the diamondback moth, *Plutella xylostella*) caught at different times of day on white sticky traps was directly proportional to the number recorded by direct observation.

The ladybird beetles *Coccinella transversalis* and *Adalia bipunctata* were more attracted to yellow than to seven other colours, and preferred pure yellow to yellow-white hues (Mensah, 1997). Other ladybirds (*Coleomegilla maculata* and *Coccinella 7-punctata*) also preferred yellow, but the lacewing *Chrysoperla carnea* was trapped equally on red, green, white and yellow sticky cards (Udayagiri et al., 1997). Böckmann and Meyhöfer (2017), in a glasshouse tomato system, found that a predatory mirid (*Macrolophus pygmaeus*) was caught in higher numbers on blue sticky traps than yellow sticky traps. Ricci (1986) examined catches of ladybird beetles on yellow sticky traps which were being used to monitor pest populations in olive groves and in safflower and sunflower fields. Attached to some of these traps were infochemical (Chap. 1) lures, used to increase further their attractiveness to target pest species, but, curiously, in the oilseed crops more ladybirds were caught on unbaited traps than on those with lures. When yellow sticky traps and yellow water traps were placed horizontally on the ground in a wheat field, similar numbers of adult hover flies were caught on both, but more hover fly larvae were caught in the water traps (Bowie et al., 1999). Harwood et al. (2001a) placed 7.5 cm^2 sticky traps on the soil surface of a winter wheat crop to monitor the activity density of linyphiid spiders and their prey. In this case, the traps were painted black to resemble in colour the ground surface and *not* be attractive, since the aim was to quantify the normal traffic of these arthropods over the surface of the soil. Although small, these traps did capture significant numbers of spiders (0.6 $trap^{-1}$ day^{-1} at web sites and approximately half this value at non-web sites), and so could be useful in quantitative studies of both intra- and interspecific interactions between spiders. As estimators of population density, however, they suffer from similar deficiencies to those described for pitfall traps (Sect. 6.2.1), and would be

best used in conjunction with other techniques, such as mini-quadrats. Sticky traps and quadrats were seen as complementary (Harwood et al, 2001a), in that the former measure cumulative activity density over 24 h (including, therefore, the night) and are efficient at catching flying insects, whereas the latter provide a measure of the absolute densities of invertebrates (including those that are inactive and hiding under soil and stones, etc.) at a single point in time, but miss many flying or fast-moving arthropods that escape during sampling.

Insects can be removed from sticky traps using white spirit (McEwen, 1997), kerosene (Mensah, 1997), toluene, heptane, hexane, xylene, ethyl acetate (Murphy, 1985), citrus oil, vegetable oil or various other solvents (Miller et al., 1993). Coloured sticky traps can be placed in most habitats, need only be replaced occasionally, and, since they are inexpensive, can be used in large numbers if the aim is to assess biodiversity and capture rare species. Colunga-Garcia et al. (1997), for example, caught thirteen species of coccinellid on yellow sticky traps.

Light traps have also been investigated as a means of monitoring abundance of predators such as adult lacewings and ladybirds (Bowden, 1981; Perry & Bowden, 1983; Honěk & Kocourek, 1986), carabid (Kádár & Lövei, 1992; Yahiro, 1997; Kádár & Szentkirályi, 1998), staphylinid (Markgraf & Basedow, 2002) and cantharid beetles (Löbner & Hartwig, 1994). If cool white light (15W fluorescent bulbs emitting light with major peaks in the visible region around 440 and 590 nm, with some UV) is used, catches of non-predatory species (such as moths) are reduced, resulting in more efficient capture and handling of predators such as lacewings and bugs (Nabli et al., 1999). It should be noted that light trap efficiency may also vary with illumination, so that changes in activity and abundance may be obscured if catches are not corrected for variations in the intensity of background illumination provided by moonlight (Bowden, 1981). Moon phases were not, however, found to affect light trap catches of ladybirds, lacewings, and nabid bugs (Nabli et al., 1999). Information is limited to nocturnally active species and trap effectiveness varies between species (Bowden, 1982). Directional light traps have been used in forests to study arthropod stratification at different heights (Basset et al., 1997). An air-inflated dirigible gliding slowly over the forest canopy, illuminated by 500 W lights and towing a large net, has also been used as an ambitious mobile light trap (Basset et al., 1997). Subaquatic light traps are available for sampling positively phototropic aquatic insects from the water mass at night (Williams & Feltmate, 1992).

Nocturnally active hymenopterans in the Parasitica are attracted to blacklight traps (emitting UV light of wavelength 320–280 nm) and these may be useful for determining relative abundance and seasonal distribution (Burbutis & Stewart, 1979). In a comparison of attraction to light of different wavelengths, ladybirds (species not given) were most attracted to blacklight traps (Nabli et al., 1999). The pyrgotid (Diptera) parasitoids of scarabaeid beetles are nocturnal and difficult to collect, but they are attracted to light. Four UV light traps were used to determine species composition and phenology of these elusive parasitoids in Texas (Crocker et al., 1996). Some species of predatory water beetle are attracted to UV and there is potential for monitoring them with blacklight traps. A solar electric module can be used to charge a 12 V battery to power the blacklight trap, and this enables the trap to be run in remote areas with minimal maintenance (Gerber et al., 1992).

Gauld and Bolton (1988) note that light trapping is a valuable collecting method for parasitoid wasps in tropical habitats, where a significant proportion of the fauna is nocturnal.

Olfactory Attraction

The McPhail trap (McPhail, 1939; Steyskal, 1977; McEwen, 1997) (Fig. 6.15), a device commonly used to monitor the numbers of olive pests such as olive fruit fly (*Bactrocera oleae*), can also be used to collect the adults of certain lacewings (Neuenschwander & Michelakis, 1980; Neuenschwander et al., 1981). The trap is suitable only for lacewing species whose adults are non-predatory (Neuenschwander et al., 1981). The attractant used is either protein hydrolysate (to which borax is added as an insect

Fig. 6.15 A McPhail trap in an olive tree canopy (M. A. Jervis)

preservative) or ammonium sulphate. Protein hydrolysate solutions, and perhaps even tryptophan solutions (van Emden and Hagen, 1976; Jervis et al., 1992; McEwen et al., 1994), might in future also be employed as attractants, in studies of lacewings and other natural enemies, for use in conjunction with visually based trapping devices, e.g., coloured sticky traps, to enhance trapping efficiency.

There is obvious scope for the use of infochemicals (Sect. 1.6) to monitor natural enemy populations in the same way as they are currently used to monitor pests (Pickett, 1988). Delta traps baited with virgin females of aphid parasitoids (Braconidae, Aphidiinae) caught large numbers of conspecific males when placed in cereal crops (Decker, 1988; Decker et al., 1993) (Fig. 6.16a). The use of pheromone traps could provide information on seasonal population trends and on the dispersal behaviour of some species of natural enemy.

Some parasitoids are attracted by the sex pheromones of their hosts (Powell, 1999; Fatouros et al., 2008). Adult female aphid parasitoids of the genus *Praon* were caught in large numbers in water traps that were combined with a source of synthetic aphid sex pheromones (Hardie et al., 1991, 1994; Powell et al., 1993) (Fig. 6.16b). Similarly, aphelinid parasitoids were collected on sticky traps baited with either synthetic sex pheromones or virgin females of the San Jose scale, *Quadraspidiotus perniciosus* (Rice & Jones, 1982; McClain et al., 1990). Sticky traps baited with Multilure, an aggregation pheromone for the bark beetle *Scolytus multistriatus*, attracted a range of parasitoid wasps which attack both eggs and larvae of the beetle (Kennedy, 1979). Other possibilities for exploiting infochemicals include the use of aggregation pheromones for monitoring Coleoptera, particularly carabid beetles (Thiele, 1977; Pickett, 1988), and the use of plant-derived chemicals as attractants. Caryophyllene ($C_{15}H_{24}$), a volatile sesquiterpene given off by cotton plants, has been used in delta traps to monitor lacewing adults and the predacious beetle *Collops vittatus* (Malachiidae) (Flint et al., 1979, 1981). Sex

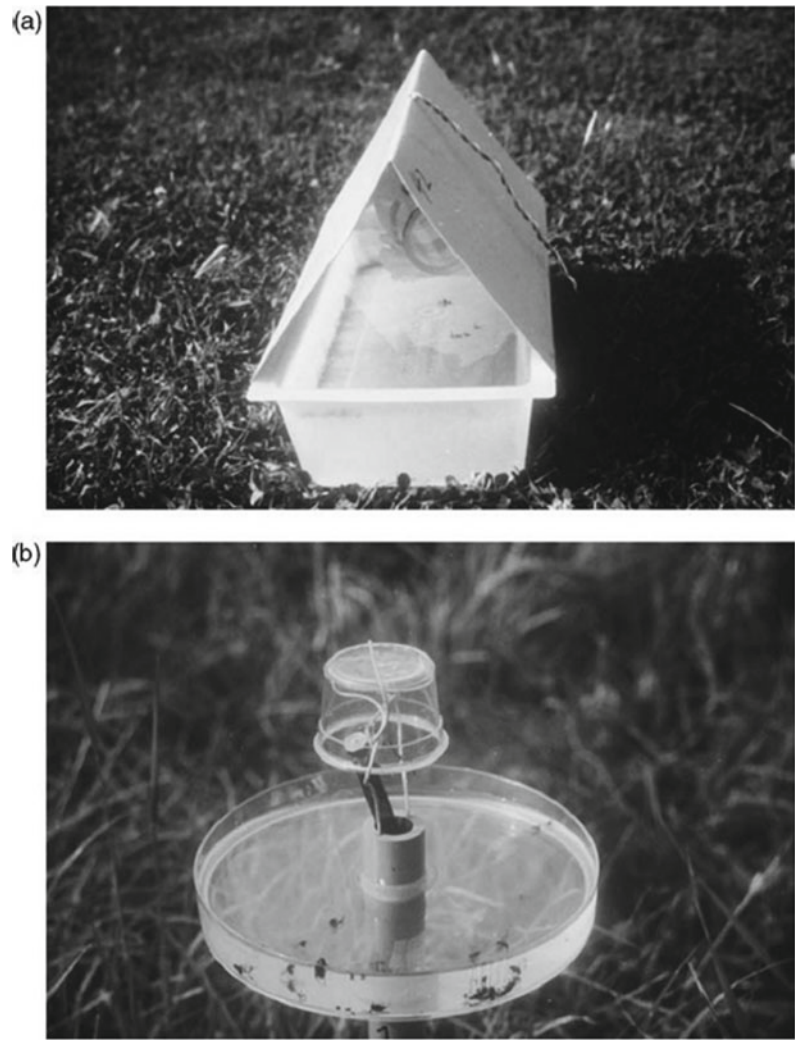

Fig. 6.16 Pheromone traps. **a** Delta-shaped pheromone trap baited with virgin female aphidiine braconid parasitoids. Captured male parasitoids that have been attracted by the female sex pheromone can be seen in the water tray. **b** Petri dish pheromone trap with aphid sex phermone lure consisting of a small glass vial containing aphid sex pheromone. Female parasitoids are attracted by the pheromone and are caught in the dish that contains water with a small amount of detergent added

pheromones of predators may also prove useful. Legaspi et al. (1996) caught females of *Podisus maculiventris* (Heteroptera, Pentatomidae) in traps that utilised the male sex pheromone of this species. Synthetic attractant pheromones have also been used to catch *Podisus distinctus* in Brazil (Aldrich et al., 1997). Predatory adult wasps, such as Sphecidae, also feed on plant nectar and are strongly attracted to commercial pest traps (e.g., white bucket traps for monitoring armyworm adults, *Spodoptera frugiperda*) baited with synthetic floral extracts (Meagher & Mitchell, 1999). Tachinid parasitoids have also shown attraction to a component of the male-produced pheromone of their stink bug hosts (Aldrich et al., 2007). Food volatiles (e.g., isobutanol and acetic acid) will attract wasps such as *Vespula germanica* and *Vespula pensylvanica* to traps that kill by drowning (Landolt, 1998), such as the Yellowjacket Trappit Dome trap (Agrisense, Fresno, California). This is a plastic trap similar in shape to the McPhail trap (Fig. 6.15) which has a transparent top half and an opaque yellow lower half. Wasps enter through the bottom and drown in a detergent solution to which various other compounds can be added. Vespine species vary in their responses to olfactory cues and combinations of food volatiles may be more effective attractants than single compounds (Day & Jeanne, 2001).

Opiine braconid parasitoids of tephritid fruit flies were caught in orchards in Hawaii using 7 cm-diameter yellow polypropylene balls (simulating ripe fruit). Each ball was split open and slices of ripe guava inserted, then the ball was covered in sticky material. Although the effectiveness of this trap was reduced somewhat by attraction of non-target Diptera and Hymenoptera, this design of trap is considered to have potential for basic population surveys, monitoring of augmentative releases, dispersal studies and behavioural studies of host habitat finding by these biocontrol agents (Messing & Wong, 1992). Imported fire ants, *Solenopsis invicta*, were monitored in fields by placing out containers smeared with commercial dog food, ground beef or sardines. The containers were then capped and frozen for subsequent counting of ants (Lee et al., 1990; Hu & Frank, 1996; Rossi & Fowler, 2000). Morrison (2002) examined baits (a freeze-killed cricket plus sugar solution on an 8 cm-diameter plastic lid) a few hours after placement, and estimated the number of individuals of each ant species by allocating them to nine abundance classes. Commercial cat food placed on 10 cm × 15 cm white cards, and left in situ for 30 min before examination, was used to monitor ant populations in deciduous forests (Weseloh, 1993). Baits consisting of cottonwool buds soaked in 20% sugar solution, and mashed canned tuna in oil, were used to monitor ant activity in tropical upland rice (Way et al., 2002). Ants (especially *Pheidole* spp. and *Lepisiota* spp.) tend to respond more strongly to protein baits (e.g., powdered fish) than to carbohydrate baits (e.g., molasses) (Sekamatte et al., 2001). Carabid beetles were attracted to pitfall traps baited with fish or pig skin (Deng & Li, 1981), and predators of horn fly, *Haematobia irritans*, were attracted to pitfall traps baited with bovine manure (Hu & Frank, 1996).

Attraction to Sound

Sound traps, broadcasting calls of mole crickets (*Scapteriscus* species), were used to attract phonotactically orientating tachinid parasitoids (*Euphasiopteryx depleta*) of these hosts (Fowler, 1987). Each sound trap was covered with a plastic bag coated with an adhesive (Tanglefoot®), which trapped the landing *E. depleta* females. The method yielded useful data on the abundance, mobility and phenology of these parasitoids. These sound traps also attracted adults of *Megacephala fulgida*, a specialist tiger beetle predator of *Scapteriscus* spp. (Guido & Fowler, 1988). Walker (1993) used an 'artificial cricket' to attract and sample females of the tachinid *Ormia ochracea*, which is a parasitoid of field and mole crickets (*Gryllus*, *Orocharis* and *Scapteriscus*). The 'artificial cricket' consisted of an electronic sound synthesiser, an amplifier and a speaker mounted in a metal box. When songs were broadcast, flies arrived within seconds. Speaker stations only 14 m apart in an apparently uniform pasture consistently produced very different fly catches (varying 2.5-fold), but the reasons for this variation are unknown. Stucky (2016) describes a modular and portable sound trap design involving multiple oblique-side entrance funnels with large openings to trap the eavesdropping cicada parasitoid *Emblemasoma erro*, which normally stays at a sound source for only a short period of time.

Using Hosts and Prey as Bait

Hosts of parasitoids can be used as bait ('sentinels') to detect the presence and level of activity of adult parasitoids in the field. Although this method is considered under the heading of sampling by attraction, the natural enemies may not locate the hosts/prey by attraction perse.

For monitoring parasitoids of the eggs of the white stemborer (*Tryporyza* [= *Scirpophaga*] *innotata*) in Javan rice, plants from an insectary infested with 2–6 egg masses were placed out in the field for 1–5 days, then retrieved and reared out (Baehaki, 1995). Similar methods were used to study parasitoids attacking eggs of leaffolder moths (Pyralidae) (De Kraker et al., 1999) and the brown planthopper, *Nilaparvata lugens* (Fowler, 1987) in rice fields. A large number of sentinel egg mass surveys have recently been undertaken in North America and Europe to evaluate egg parasitoid communities attacking the brown marmorated stink bug (*Halyomorpha halys*) in areas where the pest is invasive

(e.g., Herlihy et al., 2016; Dieckhoff et al., 2017); however, Jones et al. (2014) found that the sentinel egg mass approach probably underestimates parasitism levels (compared to naturally present eggs) in this system, possibly due to the absence of medium- and short-range host location cues associated with artificially placed sentinel eggs. To investigate parasitoids of the eggs of linyphiid spiders, Van Baarlen et al. (1994) placed spider eggsacs in the field in lidless Petri dishes and retrieved them seven days later to rear the parasitoids out. The percentage of *Erigone* spp. eggsacs parasitised by *Gelis festinans* (Ichneumonidae) determined by this method was not significantly different from that for naturally occurring eggsacs, and it was concluded that the Petri dish introduction method is a simple and efficient means of assessing parasitism in the field (Van Baarlen et al., 1994). Ellers et al. (1998) devised a trap baited with apple sauce, yeast and *Drosophila* larvae, to monitor the braconid wasp *Asobara tabida*. Potted cereal seedlings infested with cereal aphids were used by Vorley (1986) to detect winter activity of aphid parasitoids in pasture and winter cereal crops. The pots were left in the field for 14 days after which they were retrieved, and the surviving aphids reared in the laboratory until parasitised individuals mummified. Hyperparasitoids can be investigated in the same way by exposing secondary hosts that contain primary parasitoids.

Baits (sentinel prey) can also be used to monitor the activity of predators on vegetation, on the soil surface and under the soil. Bugg et al. (1987) stapled egg masses of armyworm (*Pseudaletia unipuncta*) to radish foliage to monitor predation pressure in weedy and weed-free plots. Other researchers have used artificial prey, such as dipteran puparia (e.g., *Drosophila*, *Sarcophaga*, *Musca*) stuck onto small pieces of cardboard (Speight & Lawton, 1976; Brown et al., 1988; Burn, 1989; Carcamo & Spence, 1994; Chapman & Armstrong, 1996), freeze-killed aphids (Dennis, 1991) or tethered caterpillars, to monitor predator pressure on the ground. Brust et al. (1986a) secured larvae of pest Lepidoptera to the soil surface using a 25 cm nylon thread attached to a 25 cm-long wooden stake. They claimed no effect of tethering on the behaviour of the larvae or the predators. Weseloh (1990), however, showed that tethering doubled the predation rate by ants on larvae of the spongy moth (*Lymantria dispar*). If *in-situ* observations of the baits are carried out, either by human presence (Brust et al., 1986b), or by video surveillance (Lys, 1995), the species exerting the observed predation pressure can be determined (Sect. 6.2.6). Weseloh (1990) used tethered larvae in small cages with a narrow slit that only ants could enter, compared with uncaged tethered larvae, to determine that a fifth of the predation experienced by spongy moth larvae was caused by ants. Hu and Frank (1996) seeded artificial cowpats with eggs of the horn fly, *Haematobia irritans*, and showed that most predation of the later stages of this pest was by the red imported fire ant, *Solenopsis invicta*.

Similar methods can be used to monitor predation within the soil itself. Small plastic vials, sealed with a cap, but with small holes near the top, were buried in grassland soil. The holes were large enough to allow ants to come and go, but too small to allow escape of the 'bait' prey organisms placed inside. Mealworms as bait were used to map the vertical distribution of four ant species, and a range of other prey species were used to determine their attractiveness and vulnerability to ants. Little is known about predator–prey relationships within the soil, and so this technique may also prove to be very useful in this context (Yamaguchi & Hasegawa, 1996) (Sect. 6.3.2). Eggs of Western corn rootworm *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) were placed in the soil in mesh packets of various mesh sizes to detect predation by mites, carabids and ants. Unfortunately, many eggs were also damaged in fine-mesh control packets in the field, and so the method needs further development (Stoewen & Ellis, 1991).

An alternative to sentinel prey is the use of artificial prey. Plasticine caterpillars have been used with great success in a range of habitats to determine the presence and type of predators (e.g., Loiselle & Farji-Brener, 2002; Richards & Coley, 2007; Howe et al., 2009; Lemessa et al., 2015). It is relatively easy to identify the

diference between prey attacked by rodents, birds and chewing arthropods (Howe et al., 2009).

Attraction to Refuges

Natural enemies may be attracted to refuges (such as dark, damp, cool places where nocturnal predators can find stress-free conditions during the heat of a summer's day), or they may encounter them by random movement and then remain there. This behaviour can be exploited to trap natural enemies and thus obtain information about the presence of a species in a habitat. Since the method relies on behaviour of the natural enemy, which may vary with habitat, season, age of the natural enemy, and many other factors, it cannot be used to prove absence from a habitat, nor will it provide reliable data on relative abundance, unless carefully calibrated for the target species concerned. Mühlenberg (1993) describes 'trap stones', made of concrete or plaster of Paris, containing channels sealed by a cover plate that can be placed on the ground to trap predators. Pekár (1999), Bogya et al. (2000) used cardboard paper traps, 30 cm × 100 cm, wrapped around tree trunks to monitor overwintering spiders in orchards, and Roltsch et al. (1998) used similar traps to study spider overwintering in vineyards. Similarly, earwigs (*Forficula auricularia*) were monitored in an orchard by strapping 4 cm × 5 cm PVC tubes containing corrugated paper rolls to the trunks of pear trees (Sauphanor et al., 1993). This trap design exploits the positive thigmotax (motion or orientation in response to touch) and need for a diurnal refuge which are characteristic of earwigs (Dermaptera). Folded burlap bands (30 cm wide) tied onto tree trunks with string have been used to monitor predators of fruittree leafroller *Archips argyrospila* (Braun et al., 1990). Carabid densities in the field can be raised by the provision of corrugated iron sheets, which they use as refugia, leading to increased control of mollusc pests (Altieri et al., 1982). A similar principle has been recommended for the control of slugs in horticulture, where boards of wood within plots containing vulnerable plants are used as refuges by both slugs and carabids (Symondson, 1992). The aim here is to increase encounter rates between predator and prey, to provide refuges for the predators within the crop, and to enable slugs to be both monitored and collected from beneath the boards. In aquatic systems, substrate-filled trays, leaf packs or multi-plate samplers can be used as refuges for sampling aquatic insects (Williams & Feltmate, 1992).

6.2.9 Sampling the Immature Stages of Parasitoids

Methods for detecting the presence of the immature stages of parasitoids (eggs, larvae and pupae) occurring upon or within hosts, are usually employed when estimating the impact of parasitism on host populations (Sect. 7.3), but they are also used in studies of foraging behaviour (Sect. 1.9), parasitoid life cycles and phenologies, parasitoid fecundity (Sect. 2.3), spatial distribution of parasitism (Sect. 7.3.6), parasitoid–host trophic relationships (Sect. 6.3.5), and host physiological defence mechanisms (Sect. 2.10.5).

Parasitised and unparasitised hosts can sometimes be easily distinguished visually. Insects parasitised by ectoparasitoids can often be recognised easily by the presence of eggs and larvae on their exterior. Hosts parasitised by endoparasitoids are generally less easily distinguishable from unparasitised ones, especially during the early stages of parasitism. During the later stages of parasitism, hosts may undergo alterations in integumental colour; for example some green leafhoppers become orange or yellow in colour (Waloff & Jervis, 1987). Aphid mummies (Sect. 6.2.6) are normally a distinctly different colour from live aphids (Fig. 6.13). If parasitised hosts cannot be distinguished at all from unparasitised ones by external examination, then host dissection can be used to detect the presence of parasitoid immature stages (Sect. 2.6). For example, Fowler (1987) dissected 1,500 eggs of brown planthopper, *Nilaparvata lugens*, from rice fields, and found high levels of parasitism by *Anagrus* spp. (Mymaridae) and lower levels by *Oligosita* sp. (Trichogrammatidae). Similarly, Murphy et al. (1998) examined eggs of

the grape leafhopper (*Erythroneura elegantula*) under a dissecting microscope for presence of a developing parasitoid, *Anagrus epos* (Mymaridae). Host dissection is, however, a time-consuming activity, and, for species attacked by a complex of closely related parasitoids, identification of larvae, and particularly eggs, may prove either difficult or impossible.

The immature stages of insect parasitoids have received little taxonomic study. Some morphological information is available concerning the larvae of the following groups: Hymenoptera (Finlayson & Hagen, 1977); ichneumonoids (Short, 1952, 1959, 1970, 1978); pimpline ichneumonids (Finlayson, 1967); ichneumonine ichneumonids (Gillespie & Finlayson, 1983); braconids (Ĉapek,); eurytomids (Roskam, 1982; Henneicke et al., 1992); aphidiine braconids (O'Donnell, 1982; O'Donnell & Mackauer, 1989; Finlayson, 1990); pipunculids (Benton, 1975: several genera; Albrecht, 1990: *Dorylomorpha*; Jervis, 1992: *Chalarus*); and Rhinophoridae (Bedding, 1973). In a few groups, some species can be distinguished on the basis of structural differences in their eggs, e.g., *Eurytoma* spp. Claridge and Askew (1960) and Rhinophoridae (Bedding, 1973). If one takes a large taxonomic group, such as the family Tachinidae, published descriptions of the immature stages are available for several species. There is, however, a dearth of synthetic taxonomic studies that provide identification keys.

The colour and form of aphid mummies, the shape of the parasitoid exit hole, and characteristics of the meconial pellets (waste products deposited by parasitoid larvae prior to pupation), can be used to identify the parasitoids involved, at least to genus (Johnson et al., 1979; Powell, 1982). Certain parasitoids which attack hosts feeding in concealed locations within plant tissues often leave clues to their identity, such as exuviae or meconial pellets, within the hosts' feeding cells.

Host dissection is less reliable as a method for the detection of parasitoid immatures in cases where small parasitoid stages occur inside relatively large hosts, and accuracy can vary considerably between different researchers performing dissections (Wool et al., 1978). Therefore, the most popular method for the detection of parasitoids in a sample of hosts is to maintain the latter alive in the laboratory until the adult parasitoids emerge (Sect. 6.3.6). This approach avoids the problem of identifying the parasitoid immatures but, from the standpoint of obtaining estimates of either percentage parasitism or parasitoid adult density, the problem arises of a time delay being introduced between sampling and obtaining the population estimate—parasitised hosts are removed from the influence of other mortality factors, e.g., predation, fungal pathogens, multiparasitism, host physiological defence mechanisms (Chap. 2), which would normally operate in the field after the sampling date. Therefore, the number of emerging adult parasitoids may not give an accurate estimate of the density of adults emerging in the field. The problem of estimating percentage parasitism is discussed further in Chap. 7 (Sect. 7.3.2).

The reliability of the rearing method as a means of obtaining information on the occurrence of parasitoid immatures in hosts depends upon the ease with which the hosts can be cultured. Any hosts that die during the rearing process, before parasitoid emergence is expected, should be dissected and examined for the presence of parasitoids. Results from the dissection of larvae of alfalfa weevil (*Hypera postica*) and several species of Miridae for the presence of parasitism were compared with results obtained from the rearing-out of parasitoids. Parasitism rate, as measured by dissection, was 12–44% higher than estimates obtained by rearing. This was partly because hosts and parasitoids can be attacked by entomogenous fungi, and rearing of these doubly attacked hosts produces no parasitoids. There are, however, advantages and disadvantages to using both methods (Table 6.2). Dissection may fail to detect parasitoid eggs, but these eventually register by rearing out (which also makes it easier to identify the species of parasitoid involved) (Day, 1994).

In some cases, when attacked host stages are transparent (e.g., larvae of gall midges), the number of morphology of immature parasitoids (eggs and larvae) can be rapidly observed in living hosts using transmitted light under a

Table 6.2 Comparison of dissection and rearing methods for determining the occurrence of parasitoid immatures in hosts

Objectives	Best method	
	Dissection	Rearing
Accurate mortality data		
Parasitism and disease incidence	+	
Detects combinations: superparasitism, multiple parasitism, and parasite–disease interactions	+	
Avoids additional or confounding mortality caused by stress, inadequate food, and crowding	+	
Identification of mortality factors		
Parasitoids (adults required)		+
Disease (e.g., spores required)		+
Detects kleptoparasitoids	+	
Detects hyperparasitoids	+[a]	+
Stinging mortality		+[b]
Host-feeding mortality		+[b]
Measures other factors		
Reduced feeding by parasitised hosts		+[b]
Mortality of host in different instars and stages		+[b]
Diapause of host and parasitoid		+
Sterilisation of host	+	
Convenience and logistics		
Food for host not needed	+	
Requires less expertise (usually)		+
Work load can be delayed or extended	+[c]	
Prompt results	+[d]	
Host survival is low when reared	+	
Detection of a newly-established parasite		+[e]

Source Day (1994). Reproduced by permission of the Entomological Society of America
[a]Ectoparasitoids only, unless special methods are used. [b]Laboratory studies only, not practical for field-collected hosts. [c]If hosts are frozen. [d]Total time required is not listed because it will vary with the species. In general, dissections require a more concentrated effort, but rearing extends over a long period and is less efficient, so the time spent per parasitoid detected is likely to be similar for the two methods. [e]Because much larger sample sizes are usually possible, which increases the odds of finding scarce parasitoids, and immature parasitoids often cannot be identified to species

standard compound microscope (Sampson et al., 2006). Abram et al. (2012) used this method to evaluate levels of parasitism and superparasitism of swede midge (*Contarinia nasturtii*) larvae by the solitary endoparasitoid *Synopeas myles* in the field, and by rearing parasitised hosts containing different known numbers of parasitoid laravae, evaluated effects of different degreees of superparasitism on developmental outcomes.

An X-ray machine was used to detect successfully the gregarious endoparasitoid *Pteromalus puparum* and the solitary endoparasitoid *Brachymeria ovata* in pupae of *Pieris rapae*. Hundreds of pupae could be screened simultaneously, but the early stages of parasitism were not detected (Biever & Boldt, 1970). Using magnetic resonance microimaging, it was possible to see a larva of the braconid parasitoid

Dinocampus coccinellae within the body of a living ladybird, *Coccinella 7-punctata* (Geoghegan et al., 2000). This method may, however, be better suited for studying parasitoid development and behaviour within living hosts than for mass-screening hosts to determine the incidence of parasitism. Near-infrared spectroscopy correctly identified (80–90% success rate) which house fly puparia were parasitised by parasitoid wasps, even when parasitoids were in the early stages of development. Differences between the absorption Primary>[aut]Xie, F. spectra of parasitised and unparasitised pupae may have been due to differences in moisture, chitin or lipid compositions (Dowell et al., 2000).

For researchers who have access to gas chromatography equipment, hydrocarbon profiles can provide a reliable method for identifying parasitoids, before or after leaving the host. Geden et al. (1998) used cuticular hydrocarbon profiles to identify three species of *Muscidifurax* (Pteromalidae, parasitoids of house fly and stable fly pests) which are difficult to separate morphologically. Furthermore, they showed that specimens of *M. raptor* from five countries and three continents could all be identified by the method. The method can be successful with adult parasitoids and with pupal exuviae (Carlson et al., 1999). Techniques are described in Phillips et al. (1988) and other applications of the method are listed in Symondson and Hemingway (1997).

Electrophoretic, immunological and DNA-based techniques have been developed for detecting parasitoid immature stages within their hosts (Powell & Walton, 1989; Stuart & Greenstone, 1996; Demichelis & Manino, 1998; Greenstone, 2006; Gariepy et al., 2007) (Sects. 6.3.9, 6.3.11 and 6.3.12 provide detailed discussion). Tilmon et al. (2000) used a two-stage molecular approach for determining the rate at which three species of *Peristenus* (Hymenoptera: Braconidae) were parasitising *Lygus lineolaris* (Heteroptera: Miridae) in an alfalfa field. They used PCR (polymerase chain reaction) to target part of the mitochondrial cytochrome oxidase I (COI) gene. Using mitochondrial genes ensured that there was a high copy number per cell while the part of the COI gene chosen has low intraspecific variability. Primers were designed that amplified DNA from all three *Persistenus* species and these primers were used to detect parasitoids within *L. lineolaris* nymphs. Restriction digests of the PCR product were then carried out to determine which of the three parasitoid species had been present. This procedure took days (compared with months for the rearing-out method, Sect. 6.3.6) and also gave the researchers the flexibility to store samples until convenient for processing (Tilmon et al., 2000). Gariepy et al. (2005) further refined the detection and identification of *Lygus* parasitoids within their hosts, developing the first single-step multiplex PCR assay with species-specific primers that could detect parasitism within three days of the host being parasitised. Traugott and Symondson (2008) found that it was possible to detect parasitoids (*Lysiphlebus testaceipes*) within their aphid host (*Aphis fabae*) as little as 5 min. after parasitism using PCR. Section 6.3.11 provides more detailed discussion of the molecular analysis of parasitoids within hosts.

DNA-based methods have also been used for the rapid identification of taxonomically difficult parasitoids, such as *Trichogramma* species (Menken & Raijmann, 1996; van Kan et al., 1996; Stouthamer et al., 1999; Chang et al., 2001 Sumer et al., 2009) and *Muscidifurax* species (Taylor & Szalanski, 1999), and, similarly, for predators such as phytoseiid mites (Navajas et al., 1999; Okassa et al., 2010). Sumer et al. (2009) present an identification key for ten common species of *Trichogramma* in the Mediterranean based on PCR and subsequent restriction fragment-length polymorphisms of ITS-2 fragments. RAPD-PCR assays of the encyrtid parasitoid *Ageniaspis citricola* suggested that it may be two species, one occurring in Thailand and the other in Taiwan (Hoy et al., 2000): such information can be crucial to the success of biological control programmes.

6.2.10 Mark-Release-Recapture Methods for Estimating Population Density

Many of the sampling methods discussed above provide only relative estimates of predator and parasitoid population densities, and of those that provide absolute estimates, some only work well for conspicuous or less active species. Inconspicuous or very active natural enemies, such as carabid beetles and lycosid spiders, are more difficult to count, and an alternative method of estimating population levels is to estimate densities using mark-release-recapture data. These data are obtained by live-trapping a sample of individuals from the population, marking the insects so that they can be distinguished from uncaptured individuals, releasing them back into the population, and then re-sampling the population. An absolute estimate of population density can be calculated from the proportion of recaptured, marked individuals in the second sample, provided that a number of assumptions are satisfied. The key assumptions are:

1. That marking neither hinders the movement of an individual nor makes it more susceptible to predation or any other mortality factor.
2. That marks are retained throughout the trapping period.
3. That marked and unmarked individuals have an equal chance of being captured.
4. That, following release after marking, marked individuals become completely and randomly mixed into the population before the next sample is taken.

The original method of estimating total population size from mark-release-recapture data was devised by Lincoln (1930) for the study of waterfowl populations. The standard Lincoln Index formula is:

$$N = \frac{an}{r} \qquad (6.1)$$

where N is the population estimate, a is the number of marked individuals released, n is the total number of individuals captured in the sample, and r is the number of marked individuals captured.

The Lincoln Index relies upon the sample of marked individuals that is released back into the population becoming diluted in a random way, so that the proportion of marked individuals in a subsequent sample is the same as the proportion of marked individuals originally released within the total population. The original Lincoln Index method assumes, unrealistically, that the population is closed, with no losses or gains either from emigration and immigration or from deaths and births, during the period between the taking of consecutive samples. Since the Lincoln Index was devised, a number of modifications and alternative methods of calculating population density have been developed. The best known are those of Fisher and Ford (1947), Bailey (1951, 1952), Craig (1953), Seber (1965), Jolly (1965), Parr (1965), Manly and Parr (1968), Fletcher et al. (1981). These methods, together with the formulae used to estimate population sizes, are described and discussed in detail by Seber (1973), Begon (1979, 1983), Blower et al. (1981), Pollock et al. (1990), Sutherland (1996), Krebs (1999), Southwood and Henderson (2000). Begon (1983) reviews the use of Jolly's method, based on 100 published studies, and discusses potential alternatives. The computer programme 'Capture' can be used to select the most appropriate model for a specific set of circumstances (Otis et al., 1978; White et al., 1982; Samu & Kiss, 1997; Kiss & Samu, 2000). A practical problem that is often encountered with carabids (Ericson, 1977; Kromp & Nitzlader, 1995; Samu & Sárospataki, 1995b) and lycosids (Samu & Kiss, 1997) is that, in spite of a large expenditure of effort devoted to marking individuals, the recapture rate of some species can be less than 10%. Begon (1979) provides tables of minimum sample sizes of marked and recaptured individuals for stated levels of accuracy of the population estimate.

Among natural enemies, carabid beetles are the group most often subjected to mark-release-recapture studies, but mainly for the purpose of investigating activity and dispersal (Sect. 6.2.11).

There have also been several attempts at estimating carabid (Ericson, 1977, 1978; den Boer, 1979; Brunsting et al., 1986, Nelemans et al., 1989; Hamon et al., 1990; Thomas et al., 1998), staphylinid (Frank, 1968) and lycosid (Greenstone, 1979; Kiss & Samu, 2000) population densities using mark-release-recapture data. In a ten-year study of the carabid *Nebria brevicollis*, 11,521 beetles were individually marked using a branding technique (Nelemans et al., 1989) (marking methods are discussed more fully in Sect. 6.2.11). The beetle population was sampled continuously using pitfall traps, and two methods were used to calculate population sizes: Jolly's method, as modified by Seber (1973), and Craig's method (Craig, 1953). Of the two, Craig's method gave the better estimates, those obtained using Jolly's (1965) method being much too low. All the methods used to estimate population sizes from mark-release-recapture data assume constant catchability over the trapping period, but Nelemans et al. (1989) found significant between-year differences in recapture probabilities. Also, the frequency distribution of recaptures deviated significantly from that predicted by a Poisson distribution, more beetles than expected failing to be recaptured and more than expected being recaptured three or more times. Consequently, there is a danger of significant errors occurring in estimates of carabid numbers based on mark-release-recapture data from pitfall trapping, Jolly's method in particular tending to underestimate populations (den Boer, 1979; Nelemans et al., 1989). The size of errors arising from variability in the chances of recapture will depend to some extent on the species being studied. Since the behaviour of individuals affects catchability in pitfall traps, it is sometimes advisable to treat the sexes separately (Ericson, 1977; Samu & Sárospataki, 1995b; Samu & Kiss, 1997). Furthermore, the handling procedure during marking and release will affect the activity of beetles following release. This can be mitigated, to some extent, by leaving an interval of a few days between successive trapping periods to allow marked beetles time to redistribute themselves within the population before being recaptured (Ericson, 1977).

Because carabid beetles are highly mobile, some marked individuals are likely to leave and re-enter the trapping area, thereby biasing population estimates (Ericson, 1977). To avoid this problem, enclosures have been used during some carabid mark-release-recapture studies (Loreau, 1984a; Brunsting et al., 1986; Nelemans et al., 1989) (Sect. 6.2.1). In a similar way, a field cage was used to assess the accuracy of Jolly's method for estimating populations of the ladybird beetles *Coccinella californica* and *Coccinella trifasciata* in alfalfa and oat crops (Ives, 1981). Beetles were captured in experimental plots using a visual searching method; these were marked at the site of capture with spots of enamel paint, and were then released immediately after marking. Estimates of population density were calculated using Jolly's method and, in the caged plots, these proved to be very accurate, although estimates of populations in open plots were likely to be less precise. When there was no limitation on flight, it was considered necessary that aphid densities in the study area should be high enough to provide adequate food for the ladybirds, thus preventing dispersal. A limitation noted in Ives' study was the amount of time spent marking captured beetles, which restricted the numbers that could be caught in a sampling period, thereby reducing the accuracy of the mark-release-recapture method. To alleviate this problem, visual counts were made whilst walking through the plots, in addition to the counts made whilst marking. A similar method was used to estimate wolf spider population densities in an estuarine salt marsh (Greenstone, 1979). As an alternative to enclosure, records of the distance moved by marked predators within a trapping grid (e.g., a grid of pitfalls) can be used to calculate the 'area of influence' of traps or grid (Kuschka et al., 1987; Franke et al., 1988). Area of influence can also be estimated by defining concentric rectangles within the grid and noting at which size of rectangle the density is stabilised (Loreau & Nolf, 1993). Population size (from

mark-release-recapture) divided by area of influence provides an estimate of population density.

Some mark-release-recapture studies have been carried out on hover fly populations, but on phytophagous and saprophagous rather than predatory species (Neilsen, 1969; Conn, 1976). Adult narcissus bulb flies, *Merodon equestris*, were marked on the tibiae with cellulose paint applied with a fine blade of grass (Conn, 1976), a method that could also be used with aphidophagous syrphids. In this study, population size was estimated using Jolly's method and the Fisher–Ford method. Despite recapture rates of 30–50%, both methods gave large errors in daily estimates of population size because samples were small, and the total amount of variation in estimates using Jolly's method was usually two to three times higher than that obtained by the Fisher–Ford estimates. Multiple regression analyses revealed that 35–40% of the variation in the Fisher–Ford estimates and 35–45% of the variation in the Jolly estimates was attributable to the effect of variation in temperature.

Adult parasitoids are much more difficult to mark for mark-release-recapture studies because many of them are small-bodied and difficult to handle. Some success has been achieved by labelling braconids, mymarids and aphelinids with fluorescent dust or trace elements (Jackson et al., 1988; Jackson & Debolt, 1990; Hopper & Woolson, 1991; Corbett et al., 1996; Bellamy & Byrne, 2001; Wanner et al., 2006). In some cases, the trace elements were added to artificial diets on which the hosts of the parasitoids were reared. Fleischer et al. (1986) studied patterns of uptake and elimination of rubidium by the mirid bug *Lygus lineolaris* by spraying mustard plants with varying rates of Rb. A concentration of 200 ppm RbCl added to the diet of its *Lygus* spp. hosts provided Rb-labelled *Leiophron uniformis* which could be distinguished from wasps collected from field populations for 6–8 days (Jackson & Debolt, 1990). Similarly, *Microplitis croceipes* adults labelled with rubidium or strontium via the diet of their hosts, *Helicoverpa* spp., at a concentration of 1000–2000 ppm, were distinguishable from field-collected, unlabelled wasps for up to 20 days (Hopper & Woolson, 1991). There is a danger, however, that high levels of these elements could affect the biology of the labelled insects. Jackson et al. (1988) noted that labelling *Anaphes ovijentatus* (Mymaridae) with high doses of rubidium (1000 ppm RbCl), via the eggs of its *Lygus* spp. hosts, tended to reduce longevity and fecundity slightly. Strontium was found to be superior to rubidium for labelling parasitic Hymenoptera because: (a) background levels are lower, (b) elimination rates are less, (c) it does not affect parasitoid development and behaviour, and (d) it is less expensive (Gu et al., 2001). The radioisotope ^{32}P was used to label *Trichogramma dendrolimi*, a parasitoid of lepidopteran eggs, in order to evaluate the impact of mass releases against a tortricid moth (Feng et al., 1988). In this case, no adverse effects of the radioisotope labelling on longevity, reproduction or sex ratio of the parasitoid were detected. Similarly, Goubault and Hardy (2007) found no effect of heavy water (deuterium oxide) marking (via development on hosts injected with the marker) on the lifetime reproductive success of *Goniozus legneri*, making it a promising candidate marker for mark-release-recapture studies as well as laboratory studies of real-time chemical volatile emissions associated with competition. This method was also shown to be cost-effective, and more sensitive than other labelling methods.

It is also possible to label parasitoids indirectly by applying stable isotopes to host plants. For example, Wanner et al. (2006) showed that applying aqueous drenches containing the rare stable calcium isotope ^{44}Ca to cabbage plants resulted in transfer of the isotope to parasitoids (*Cotesia glomerata*) developing in caterpillars (*Pieris brassicae*) that had been reared on the isotope-drenched plants, without effects on host or parasitoid development or survivorship. Greenhouse experiments showed that the isotope could then be transferred to hosts by labelled parasitoids, providing a method to measure dispersal and foraging behaviours of parasitoids without the need to recapture released individuals. A drawback of this method,

however, is that analysis of ^{44}Ca-labelled samples using stable isotope analysis requires a large amount of biomass (often necessitating pooling of samples), which can be problematic for smaller parasitoid species.

By applying unique marks to each individual insect in a mark-release-recapture study, additional information on longevity and survival rates can be obtained. Conrad and Herman (1990) used data from a mark-release-recapture study of dragonflies to calculate daily survival estimates using the Manly-Parr method (Manly, 1971; Southwood & Henderson, 2000), which were then converted to daily expected life span estimates using the formula of Cook et al. (1967): Expected life span = $-1/log_e(survival)$. Conn (1976) calculated the longevity of phytophagous hover flies from mark-release-recapture data.

6.2.11 Methods Used in Investigating Natural Enemy Movements

Predators need to locate their prey in order to feed, and parasitoids must find their hosts in order to lay eggs on/in them. Biocontrol practitioners need to determine how far parasitoids can move between host areas and areas of non-host foods to decide, for example, where to site plants for the supply of pollen and nectar (Chap. 8). Prey/host location generally requires spatial displacement on the part of the predator or parasitoid, and this may be either directed (e.g., movement towards the source of a stimulus) or random. Such locomotor activity is a major factor influencing the population dynamics of natural enemies because it affects the rate of encounters with prey and host patches and so influences the amount of predation or parasitism. It occurs as a result of behavioural responses to stimuli which may be internal, physiological stimuli, e.g., hunger (Mols, 1987), or external, environmental stimuli, e.g., infochemicals (Sect. 1.6). In the main, host- and prey-location behaviour involving spatial dispacement constitutes trivial movement, i.e., it is restricted to the habitat normally occupied by the insects, whereas migratory movements take the insect away from its original habitat (Southwood, 1962), although there are notable exceptions such as some mymarid wasps. The techniques described here can be applied to either type of movement, although so far as trivial movements are concerned, foraging behaviour at a low level of host and prey patchiness is not dealt with (Chap. 1 discusses this aspect of behaviour).

The study of insect movement in the field is often difficult; in general, it is easier to measure the consequences of movement than it is to examine the process itself. For example, it is easier to record that an individual has shifted from one location to another during the period between two sampling occasions than it is to record the path taken by that individual, or the speed at which it travelled.

Some measure of the level of locomotor activity within a population can be obtained by intercepting moving individuals. For a given population, the higher the level of activity, the more individuals will be intercepted within a given time. Pitfall traps, for example (Sect. 6.2.1), can be used to study the activity of ground-dwelling predators, such as carabid beetles. In order to study the diurnal activity patterns displayed by carabid beetles and other surface-living predators, automatic, time-sorting pitfall traps have been developed and used in a variety of habitats (Williams, 1958; Ayre & Trueman, 1974; Barndt, 1976; Luff, 1978; Desender et al., 1984; De Keer & Maelfait, 1987; Alderweireldt & Desender, 1990; Kegel, 1990; Bayram, 1996). Individuals falling into the trap are channelled into fresh collecting tubes or compartments after predetermined time intervals, which may be as short as two hours (Ayre & Trueman, 1974; Kegel, 1990). Funke et al. (1995) used time-sorting pitfall traps at the tops of two-metre-high artificial tree trunks to investigate the diel periodicity of flying predators in deciduous woodland. These authors also devised ring-shaped pitfall traps, and deployed them, with and without artificial tree trunks in the middle, to determine which species of predator (carabid and

staphylinid beetles, spiders, harvestmen and centipedes) use trunk silhouettes for orientation when moving on the ground.

Population survival can depend on the rate of exchange of individuals between sub-populations. For non-flyers this exchange is by walking. To measure relative rates of dispersal by walking, five species of carabid were individually marked and released in the centre of 7 m-radius fenced areas, then caught in pitfalls at the edge. Circles were set up in areas of high- and low-density vegetation (Klazenga & De Vries, 1994). Similarly, a 10 m-diameter circle of pitfalls was placed such that half the circle was in an oat field and half in adjacent grassland. Carabids were captured and marked with coloured paint (a different colour for each habitat), then released. 11% were recaptured and about 4% were recaptured in a different habitat from the one where they were originally caught (Kajak & Lukasiewicz, 1994). The same approach, using 10 m-diameter enclosures, was employed to investigate the effects of insecticides on the movement of several carabid species (Kennedy et al., 1996; Kennedy & Randall, 1997). Circles of 10 m–20 m radius were used by Ellers et al. (1998) who released marked braconid parasitoids (*Asobara tabida*) in the centre of the circle and investigated dispersal ability in relation to the body size of individual wasps. Chapman and Armstrong (1996) employed pitfalls, together with time-specific enclosure of some plots with plastic barriers, and deduced that some carabid beetles moved from intercropped to monocropped plots at night. This methodology showed that, at night, the carabid *Pterostichus melanarius* will move a short distance out from dense vegetation into adjacent open crops, which could justify the use of grass strips and weed strips for enhancing biocontrol within fields (Chapman et al., 1999). Linear pitfall traps can also be used to gain information on direction of movement (Duelli et al., 1990). If such traps are positioned along the boundary between two distinct habitats, they will detect major population movements across the boundary, and this could be related to changing conditions, such as levels of prey availability, within habitats. Linear traps placed at increasing distances from a habitat boundary will give some indication of rates of immigration into that habitat, e.g., rates of colonisation of arable fields from overwintering refuges (Pausch et al., 1979). Petersen (1999) sited a vertical polyethylene barrier in an arable field parallel to the field edge; the barrier was set in a zig-zag pattern and pitfalls were sunk in corners of the barrier on the edge side to record spring emigration of the carabid, *Bembidion lampros*, from overwintering sites in a grass bank. Fagan (1997) studied the movement of two species of mantid out of experimental plots. The plots were surrounded by sticky plastic sheets which acted as absorbing boundaries. The pattern of emigration for *Mantis religiosa* was stepped and a 'boundary-flux' diffusion model was inappropriate, but cumulative dispersal of *Tenodera sinensis* was sigmoidal and the diffusion model provided a good fit. The model assumes that all individuals are identical, exhibit Brownian random movement at a constant rate, and that the area is homogeneous (Fagan, 1997). Diffusion models have been used to predict the effectiveness of agroecosystem diversification for enhancing natural enemies. The spatial scale at which enhancement occurs is influenced by mobility (represented by the diffusion coefficient) of the natural enemy (Corbett & Plant, 1993). Coloured water traps placed, for example, at different distances from a field edge, may enable the distribution of flying predators and parasitoids to be mapped (Hradetzky & Kromp, 1997).

Interception traps, such as window traps and sticky traps, can be used to monitor the activity and movements, including the direction of movement and height above ground, of flying insects. A window trap consists of a sheet of transparent material, such as glass or plastic, supported in a vertical position, at an appropriate height above the ground, by means of a rigid frame (Fig. 6.17a). Flying insects collide with the window and fall into a water-filled tray fixed to its lower edge. Lengths of plastic guttering, fitted with end stops, make convenient trays. A drainage hole bored into the base of the tray, and closed by means of a rubber or plastic bung,

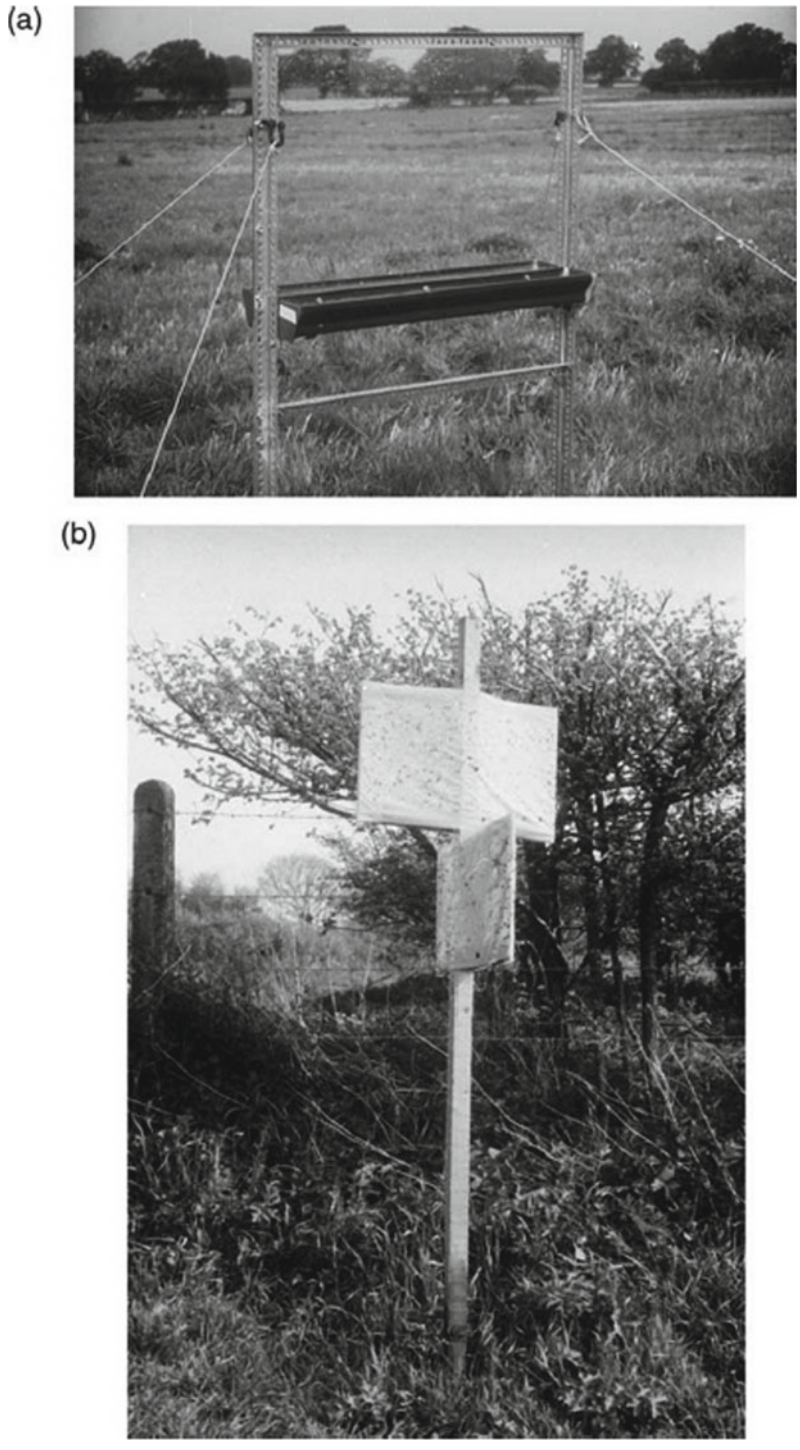

Fig. 6.17 Traps for interception of flying insects: **a** window trap; **b** sticky trap. The window trap consists of a sheet of transparent plastic; the insects are caught in sections of guttering containing water with a small amount of detergent

allows it to be emptied at the end of each sampling period. Catches can then be sorted in the laboratory. The 'vane-style' window trap (made from two intersecting pieces of clear PVC plastic arranged over a funnel and collecting vessel) can be set on the ground or hung in the canopy of a tree (Juvonen-Lettington & Pullen, 2001). Separate trays are normally fitted to each side of standard window traps to provide information on flight direction. Directional information can be increased by using two traps positioned at right angles to one another. Data from window traps have been used: (a) to relate the flight activity of carabid beetles to wind direction and to the reproductive state of females (van Huizen, 1990), (b) to monitor the immigration of carabids into newly-formed polders in the Netherlands, and thereby detect the establishment of new populations (Haeck, 1971), (c) to monitor levels of predator activity in cereal fields (Storck-Weyhermüller, 1988), and (d) to detect periods of aerial dispersal by spiders (De Keer & Maelfait, 1987), carabid beetles (Markgraf & Basedow, 2000) and staphylinid beetles (Markgraf & Basedow, 2002). Hance (1995) used directional window traps to show that more ladybirds, *Coccinella 7-punctata*, were entering a maize field from an adjacent orchard than from an adjacent fallow field. He also noted that some carabid species (such as *Demetrias atricapillus* and *Bradycellus verbasci*) were well represented in window traps but were very rarely caught in pitfall traps in the same area. Thus, window traps are an important supplement to pitfalls for documenting the carabid fauna of a region. Window traps can also be hung from the trunks of trees to intercept strong-flying arthropods, such as wasps and predatory beetles that are flying towards the tree (Hanula & Franzreb, 1998). Stenbacka et al. (2010) compared the use of trunk window traps to trunk emergence traps in estimating species richness, abundance, and community composition of ichneumonid parasitoids in forest stands. They found that window traps gave better measures of species composition in different forest stand types, but that trunk emergence traps gave more information about phenology, substrate requirements, and host species use. Another type of flight-interception trap, used to monitor the movement of staphylinid beetles in a Canadian raspberry plantation, comprised 1.8 m^2 window screen mesh (1.5 mm) held over galvanised metal pans containing 2% formalin solution (Levesque & Levesque, 1996). Boiteau et al. (1999) constructed a 15 m-high sampling tower holding 40 interception traps (10 per side), placed at regular intervals above ground. Each trap was a plywood board, painted yellow, with an antifreeze-filled gutter below. The tower was sited in a pasture in an agricultural area and showed that, for thirteen species of ladybird, more than half the flights were at or below 3.8 m. The tower was also used to monitor aerial dispersal and vertical flight profiles of other predators, including Carabidae, Staphylinidae and Neuroptera (Boiteau et al., 2000a, b).

Sticky traps work on a similar principle to window traps but consist of a sticky surface to which the intercepted insects adhere upon impact (Fig. 6.17b). Any suitable surface, either flat or curved, can be coated with a weatherproof adhesive, several types of which are commercially available. Sticky traps supported on poles at various heights were used to monitor the activity of predatory anthocorid bugs in pear orchards (Hodgson & Mustafa, 1984). Very few anthocorids were caught on traps placed within the orchard, but those placed at its edges successfully detected two peaks of flight activity during the year, and the peaks are believed to represent movement to and from hibernation sites. The airborne dispersal of the predatory mite *Phytoseiuliis persimilis* and its prey *Tetranychus urticae* was investigated using sticky traps made from microscope slides coated with silicon grease (Charles & White, 1988). These were clipped in a vertical position onto pieces of wooden dowelling, which were arranged in the form of a horizontal cross at the top of a supporting pole. Asplen et al. (2016) used sticky traps (rectangular pieces of cut transparency film coated with spray adhesive) placed at different heights with respect to the canopy of soybean plants to measure the dispersal of the parasitoid *Binodoxys communis*. Using this method, they were able to show sex-specific differences in

dispersal characteristics; for example, female parasitoids were more likely than males to climb into higher air columns above the canopy and engage in wind-assisted dispersal.

A network of 29 yellow sticky traps was used to study dispersal of coccinellid beetles on a 330 ha farm. The study showed that *Propylea 14-punctata* and *Adalia bipunctata* appeared first on traps placed near grass banks and hedgerows, but *Coccinella 7-punctata* was more evenly distributed (Zhou et al., 1994). Greenstone et al. (1985) compared horizontal sticky wires with vertical sticky panels for monitoring ballooning spiders above a soybean field. Wire traps underestimated numbers of small money spiders (Linyphiidae). Traps subsequently used (Greenstone, 1990) comprised 16 sheets of 25 cm × 50 cm, 12.7 mm mesh, galvanised hardware cloth, coated with adhesive, and stapled to a wooden frame. They were hung by clamps from parallel horizontal pairs of clothes lines attached to vertical posts set in the ground. This design enabled rapid replacement of traps, which was done weekly. In some habitats sticky traps can also be useful for monitoring natural enemies that are walking or running, rather than flying. Docherty and Leather (1997) attached two polythene bands, covered with sticky Oecotak™, to the trunks of pine trees to estimate the numbers of spiders and harvestmen (Opiliones) ascending and descending the tree trunks. Van Laerhoven and Stephen (2002) suspended a series of sticky-coated wire-mesh traps from a rope at 4 m intervals to a height of 16 m on pine tree trunks to catch foraging adult parasitoids of the Southern Pine Beetle *Dendroctonus frontalis*. They noted that, for maximum efficiency, traps should be in contact with the trunk because parasitoids disperse by walking as well as flying. Braun et al., (1990) applied 10 cm bands of sticky Tanglefoot® directly to the trunks of trees to monitor movement of the natural enemies of fruittree leafroller, *Archips argyrospila*. Directional arboreal photoeclectors (Sect. 6.2.7) can also be used to study upward or downward migration of arthropods on tree trunks (Basset et al., 1997).

Sticky trap catches of parasitoids and predators can sometimes be increased by using colour as an attractant (Neuenschwander, 1982; Moreno et al., 1984; Trimble & Brach, 1985; Samways, 1986; Antolin & Strong, 1987) (Sect. 6.2.8), but it is questionable whether meaningful information on natural patterns of movement can be obtained using coloured sticky traps, because of the likelihood of the traps' attractant properties interfering with the natural behaviour of insects. The material comprising interception traps, even colourless, translucent ones, should be tested for its degree of UV reflectance, if such traps are to be used for investigations of insect movements.

The direction and height of flight of green lacewings in alfalfa fields was recorded using wire-mesh sticky traps attached to poles, as were data on both diel and seasonal patterns of flight activity (Duelli, 1980). Similarly, wire-mesh traps, measuring 1 m^2 and placed at a range of heights, were used to monitor insect flight activity in cereal crops (Kokuba & Duelli, 1980). The main predators caught were adult ladybirds, hover flies and lacewings, but there was evidence that different species preferred to fly at different heights, so that the choice of trap height can be very important. On a larger scale, sticky nets measuring 2.5 m high by 1.5 m wide were used to monitor the flight activity of two ladybird species around experimental plots of alfalfa and oats (Ives, 1981). Captures of marked beetles in these nets helped to demonstrate that reductions in ladybird numbers recorded in certain plots were due to the emigration of beetles, rather than to beetle mortality.

Sticky material (e.g., Tanglefoot®) can also be used in a different way to study small-scale movements of walking natural enemies. Rosenheim and Brodeur (2002) used Tanglefoot® to create funnel shapes on papaya petioles. Ladybird larvae (*Stethorus siphonulus*) detected and avoided the sticky walls of the funnel and were channelled into a holding area (delimited by a solid barrier of Tanglefoot®) on the petiole, because it was easy for them to enter by the wide end of the funnel but very difficult to exit by the narrow end. These funnel traps required only

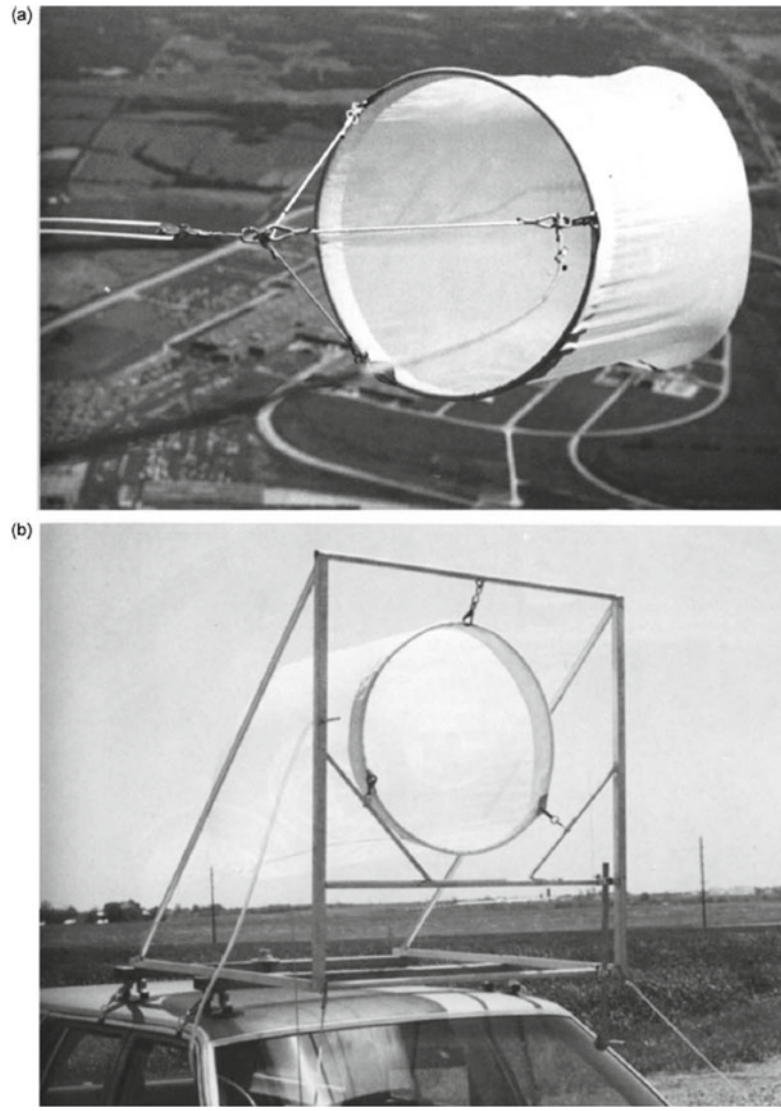

Fig. 6.18 **a** Insect net deployed in flight showing details of harness, reducing pulley, and main line weak link. **b** Frame for mounting an insect net atop an automobile. *Source* Greenstone et al., (1991). Reproduced by permission of The Entomological Society of America

minor repair over a ten-day period, including light rainfall, and enabled the authors to determine the stage during larval development at which *S. siphonulus* exits from papaya leaves.

Nets are more efficient than solid interception traps for catching weak flyers, because they interfere much less with wind flow. It was estimated that the wire-mesh sticky traps used to catch green lacewings reduced wind speeds by only 10% (Duelli, 1980). Therefore, Malaise traps constructed of netting, and the Masner-Goulet trap (Sect. 6.2.4), might be useful for monitoring the flight activity and movement patterns of insect parasitoids. The vertical distribution of aeronautic spiders and mites at heights up to 100 m was established by attaching nets to three tall radio masts (Freeman, 1946). A different approach to netting natural enemies is to employ mobile nets, which provides great versatility in the choice of horizontal and vertical sample zones. Greenstone et al. (1991) carried 0.62 m-diameter nets attached to an automobile and a light aircraft (Fig. 6.18). A slow-flying (72 km h^{-1}) fixed-wing aircraft did not destroy small or delicate natural enemies, and the pair of nets attached to such an aircraft sampled at a rate of

38,910 m^3 h^{-1} (compared with automobile sampling at 21,420 m^3 h^{-1}). Natural enemies sampled by this method included spiders, predatory flies (Dolichopodidae), wasps, ants and parasitoid wasps, staphylinid and coccinellid beetles and anthocorid bugs. Many of these were still alive when nets were emptied (Greenstone et al., 1991). Other options for flying aerial nets, for which methods have been devised, include helium-filled balloons, parafoil kites, radio-controlled model aircraft and helicopters (Reynolds et al., 1997). Currently, the use and application of unmanned 'drones' is developing rapidly (Kim et al., 2018).

Water-filled tray traps (deposition traps) were used to monitor aerial dispersal of spiders (Topping & Sunderland, 1995; Weyman et al., 1995; Thomas & Jepson, 1997, 1999). Each trap was a 10 cm deep, 1 m^2, fibreglass tray, filled with water and ethylene glycol (20:1) plus 1% detergent, fitting inside a 1.6 m^2 metal tray containing the same fluid (Fig. 6.19). The inner tray sloped towards a central hole to which a muslin tube was attached and tied. The outer tray acted as a barrier (moat) to prevent spiders walking from the crop to the inner tray, which therefore received only aerial immigrants (Topping & Sunderland, 1995). These deposition traps caught more spiders than sticky sheets of the same area laid flat on the ground (deposition:sticky was 1.9:1). Spiders in the vegetation may have snagged their ballooning lines on the edge of the deposition trap and then been able to run across the moat into the inner tray. Deposition traps have the advantage that they can be left unattended for long periods, but they are more labour-intensive to operate than rotor and suction traps (below) (Topping & Sunderland, 1995).

Suction traps (see Southwood & Henderson, 2000, for general design) have been used to determine the phenology and vertical location of ballooning spiders (Salmon & Horner, 1977; Dean & Sterling, 1990; Sunderland, 1991; Blandenier & Fürst, 1998; Topping & Sunderland, 1998; Thorbek et al., 2002), carabid and staphylinid beetles (Sunderland, 1992) and the green lacewing, *Chrysoperla carnea* (Perry & Bowden, 1983). Traps were located variously in fields, at the edge of fields, and on the tops of buildings. Holzapfel and Perkins (1969) caught spiders,

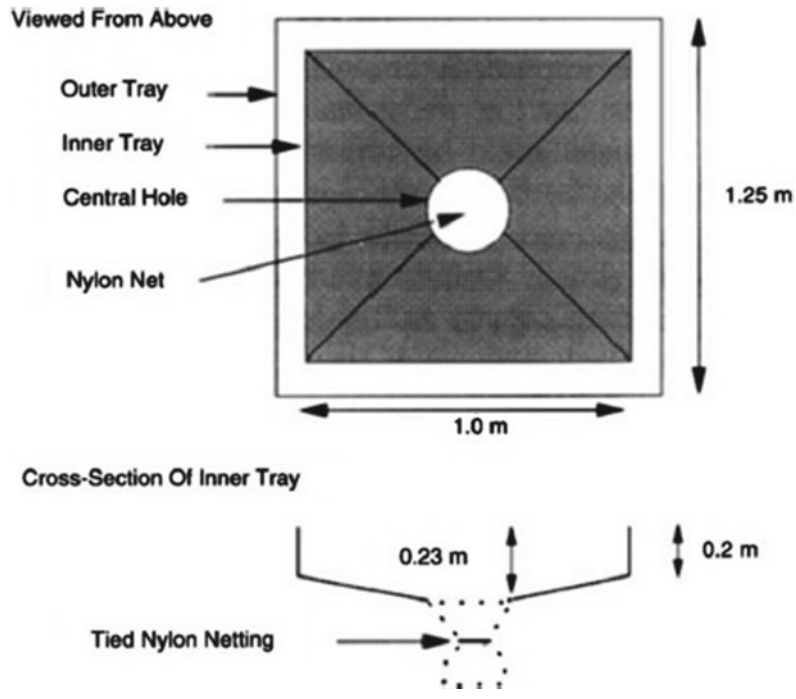

Fig. 6.19 Plan of a deposition trap. *Source* Topping and Sunderland (1995). Reproduced by permission of Aarhus University Press

ants, parasitoid wasps, staphylinid beetles and predatory Heteroptera in an electric suction trap on board ship during nine cruises across the Pacific. The traps used in these studies were the Johnson–Taylor suction trap (Johnson & Taylor, 1955), the 46 cm-diameter Enclosed-cone Propeller Suction Trap (Taylor, 1955) and the Rothamsted Insect Survey 12.2 m suction trap (Taylor, 1962). These traps have motor-driven fans that draw air down a tube, and the arthropods are segregated from the air by a filter and then deposited in a container filled with preservative. Miniature fan traps have been used to monitor the movement of parasitoids. Bellamy and Byrne (2001) deployed 102 such fan traps to study dispersal of the aphelinid *Eretmocerus eremicus*. Each trap had a 12 V DC 0.12 A fan which drew air into a 7.6 cm-long PVC pipe containing a plastic collecting vial with an organdy base; wasps were drawn into the trap and held against the base of the vial by suction. The cost of materials for such small battery-operated fan traps is about US$10, and they are ideally suited for parasitoid studies in that they capture insects intact and alive and are insufficiently powerful to catch larger insects (Hagler et al., 2002), which results in more efficient processing of the catch. Collecting vials can be quickly and easily capped, removed and replaced.

Zhang et al. (1997) caught flying carabids (*Harpalus rufipes*) in blacklight traps (Sect. 6.2.8) and found that 97% were sexually immature, suggesting a pre-reproductive dispersal phase in this species. It is likely that these beetles disperse by flight over great distances since flights of tethered beetles in the laboratory typically lasted for about two hours (Zhang et al., 1997).

Vertical-looking radar (VLR), which can automatically monitor the horizontal speed, direction, orientation, body mass and shape of arthropods intercepting the beam (Smith & Riley, 1996) could, potentially, provide useful information on the aerial migration of natural enemies and other benefical insects (Chapman et al., 2004). This technique has recently been used to investigate the dispersal of two ladybird species in the UK (Jeffries et al., 2015).

Techniques such as blacklight and Malaise traps rely mainly on the behaviour of the natural enemy. Suction traps and sticky traps tend to be sensitive to weather conditions and the size of the organism. To circumvent some of these problems, a large rotary interception trap was devised (Fig. 6.20). It had a high efficiency and had the

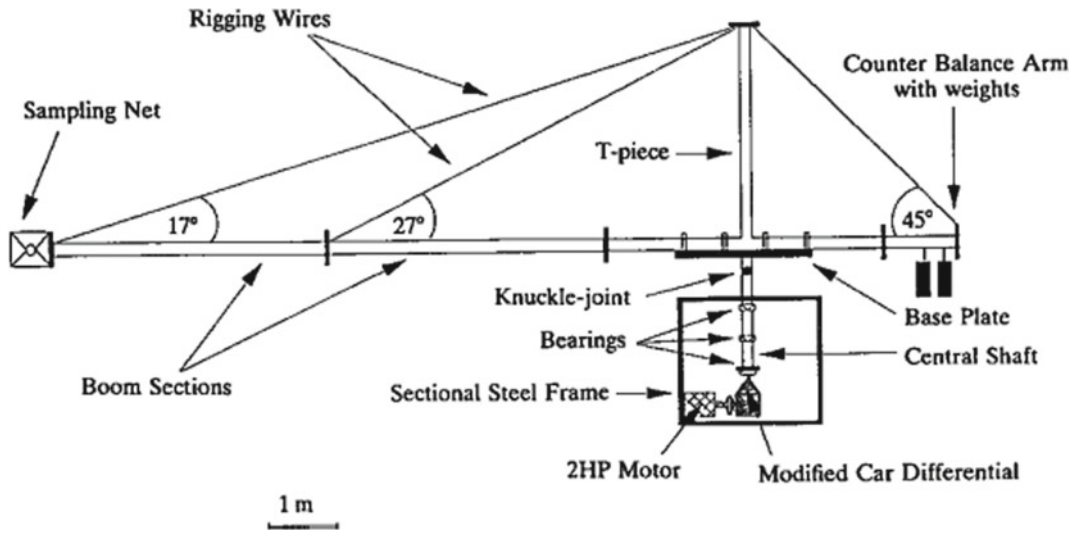

Fig. 6.20 Diagrammatic representation of a rotary sampler. *Source* Topping et al. (1992). Reproduced by permission of The Association of Applied Biologists

advantage that the sampling area was precisely defined. The rotary trap was more efficient than a co-located suction trap at catching Syrphidae, Staphylinidae and parasitic Hymenoptera (Topping et al., 1992).

If the vertical aerial distribution of natural enemies is being investigated, then it is important to bear in mind that the number of insects trapped at a particular trap elevation will be a function of both the spatial density of the insects at that elevation and the downwind component of their ground speed (Duelli, 1980). The latter depends upon the wind velocity (V_w) and the flight velocity (air speed) (V_L) of the insects. If the flight course coincides with the wind direction, the two velocities sum, but if the insects fly on a course at an angle α to the wind direction, the downwind component of the ground speed is $V_W + V_L.\cos\ \alpha$ (Duelli, 1980). If, as is likely, wind velocity increases with height from the ground (Fig. 6.21a), the numbers of trapped insects (N_T) need to be corrected for each trap elevation, to obtain the relative densities (N_F) per unit volume of air, as follows (see Fig. 6.21b):

$$N_F = N_T/(V_W + V_L cos(\alpha)) \qquad (6.2)$$

Interception traps can also be used to study the movement of aquatic predators which are carried along in flowing water (Elliott, 1970; Williams & Feltmate, 1992; Southwood & Henderson, 2000). These devices generally take the form of tapering nets, with or without a collecting vessel attached, which are either positioned on the stream bed (Waters, 1962), or are designed to float (Elliott, 1967).

Valuable information on natural enemy activity in the field can be gained by monitoring the movements of marked individuals (Scott, 1973). The commonest of the marking techniques used for this purpose is the application of spots of paint (usually enamel paint because

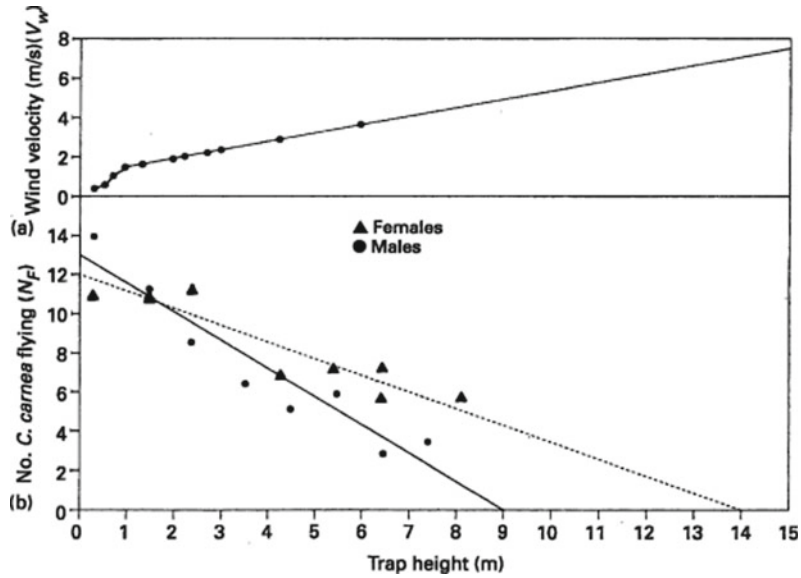

Fig. 6.21 Correcting the results of sticky trapping (traps set at varying heights above the ground) for the effects of wind velocity, in the green lacewing, *Chrysoperla carnea*, in alfalfa fields. This figure shows: **a** wind velocity (V_w, see text) as a function of height in an alfalfa field. In a night with average wind speed, the boundary layer (the layer within which the insect's air speed exceeds wind speed) is at 50 cm. Above 1 m the ground speed is mainly a function of the wind speed; **b** the relative numbers (N_F, see text) of insects flying at a particular elevation. The numbers can be estimated from the vertical distribution of trapped insects by correcting for wind speed, air speed of the lacewing, and the angle between wind direction and flight course (see text for details). The corrected vertical distribution of females in the oviposition phase and of males, are treated here as linear regressions. *Source* Duelli (1980). Reproduced by permission of Springer Verlag

some other types of paint may have toxic effects), which has been used to mark carabid beetle adults (Jones, 1979; Perfecto et al., 1986; French et al., 2001) and larvae (Nelemans, 1988), cantharid beetle larvae (Traugott, 2002), lycosid spiders (Hackman, 1957; Dondale et al., 1970; Samu & Sárospataki, 1995b; Samu & Kiss, 1997), linyphiid spiders (Samu et al., 1996), araneid spiders (Olive, 1982) and adult ladybirds (Ives, 1981). Tiny protonymphs of predatory phytoseiid mites have even been marked using watercolours (Schausberger, 1999). Acrylic paint was used to mark *Episyrphus balteatus* (Syrphidae) for short-duration dispersal studies. Individuals did not appear to be adversely affected and were seen foraging normally for 90 min after marking (MacLeod, 1999). Alternative fluid markers are inks (Kromp & Nitzlader, 1995) and typewriter correction fluid (Holland & Smith, 1999; Raworth & Choi, 2001). These marks may, however, be lost, particularly by carabid species which burrow in the soil or squeeze themselves into cracks or under stones. It is advisable, therefore, to test the durability of any marking system in preliminary trials. Raworth and Choi (2001) advocate painting identical marks on each elytron, since the probability of losing both marks (at least for short-duration studies of epigeal beetles) is very low. More robust marking systems have been used with (or are suitable for) carabid beetles, including branding with a surgical cautery needle (Fig. 6.22a, b), or a fine-pointed electric soldering iron (Manga, 1972; Ericson, 1977; Nelemans et al., 1989; Mikheev & Kreslavskii, 2000), etching the elytral surface with a high-speed drill (Best et al., 1981; Wallin, 1986; Thomas et al., 1998; Fournier & Loreau, 2002), cutting notches in the edges of the elytra and thorax with a small medical saw (Benest, 1989; Loreau & Nolf, 1993), cutting the tips off the elytra (Wallin, 1986) and etching the dorsal surface of the elytra with an engineering laser (Griffiths et al., 2001). It is important, especially when using a technique which involves physical mutilation, to ensure that marked individuals have the same survival rate as unmarked individuals and that their subsequent behaviour is unaffected.

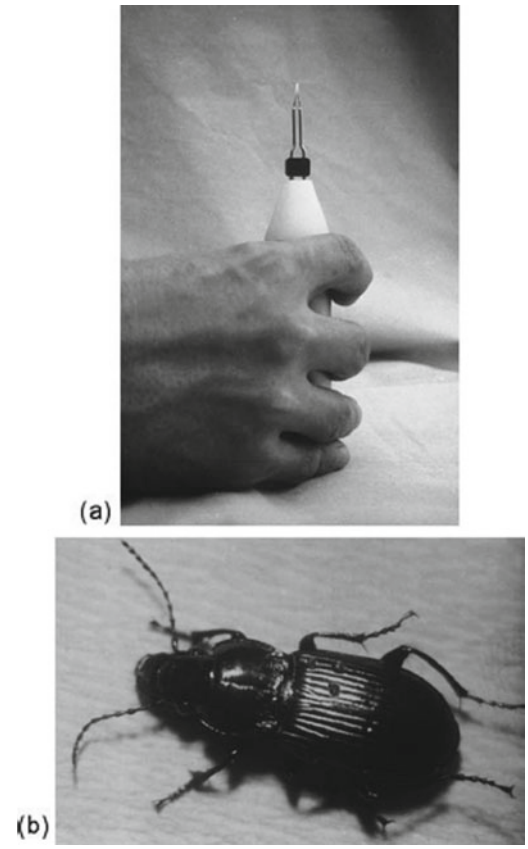

Fig. 6.22 **a** Surgical cautery needle used for marking the elytra of carabid beetles; **b** carabid beetle (*Pterostichus melanarius*) marked using the needle

A system of mark positions can be devised that will allow large numbers of a natural enemy species to be individually marked (Samu et al., 1996; Thomas et al., 1998; Holland & Smith, 1999; Southwood & Henderson, 2000). For example, Kiss and Samu (2000) marked the dorsum of the cephalothorax and abdomen of lycosid spiders with dots of enamel paint in such a way that the trap in which the spider was collected was coded by position of the dots, the date of trapping was coded by colour, and a supplementary dot was added if the spider was recaptured.

Information on minimum distances travelled and speed and direction of movement can be obtained from the recapture data of marked individuals (Evans et al., 1973; Ericson, 1978; Greenstone, 1979; Best et al., 1981; Nelemans,

1988; Mader et al., 1990; Lys & Nentwig, 1991; Zhang et al., 1997; Thomas et al., 1998). Evans et al. (1973) studied the movements of adult mutillid wasps in an open sandy habitat. The area was divided into a grid using wooden stakes and patrolled daily by an observer. Adult wasps were caught, marked with spots of coloured paint (of the sort used for model aircraft) using the head of an insect pin, and then released. Individuals could be recognised by the colour and positions of paint spots. Similarly, valuable data about the dispersal behaviour of parasitoid wasps attacking a tephritid fly on thistles were obtained by paint-marking (Jones et al., 1996). Jones et al. marked adult parasitoids with acrylic paint, and the location of individuals on thistles within a study site was recorded daily. Laboratory tests showed that marking reduced longevity of one species slightly, but the others were unaffected. The average recapture rate was 22%. The activity of wolf spiders in an estuarine salt marsh was investigated in a similar way; the spiders were caught using an aspirator and individually marked with enamel paint (Greenstone, 1979). During an investigation of the population dynamics of the dragonfly *Calopteryx aequabilis*, Conrad and Herman (1990) studied movement patterns in relation to adult territoriality by marking insect wings with a drawing pen so that individuals could be recognised. The study was carried out along a 635 m section of a stream, which was divided into 5 m sectors, delineated with small numbered flags. Observers patrolling the stream noted the sex, age category and sector-location of all individuals sighted. Population movements were analysed using a modification of Scott's (1973) method, which allows separation of the velocity and distance components of movements.

Marking can be dispensed with in cases where the species to be studied is new to an area. For example, Coll and Bottrell (1996) studied the eulophid *Pediobius foveolatus*, which parasitises Mexican bean beetle (*Epilachna varivestis*, Coccinellidae), and does not overwinter in Maryland where the studies were conducted. The authors released parasitoids at the edges of field plots, and, after various periods of time (depending on the experiment), they vacuumed all plants in the plots to record the movement of the parasitoids (Coll & Bottrell, 1996). In an analogous study, *Lysiphlebus cardui* (Braconidae, Aphidiinae), adults were released in a mountain area of Germany before the natural populations were active. Potted thistles containing colonies of the host aphid (*Aphis fabae cirsiiacanthoidis*) were distributed at up to 470 m from the release point, but female *L. cardui* were found to move a maximum of 20 m to a new host patch (Weisser & Völkl, 1997). In the context of the release of new species of parasitoid, hosts as 'bait' (Sect. 6.2.8) can be used to monitor the progress of dispersal. 'Sentinel' egg masses of the spruce budworm (*Choristoneura fumiferana*) were placed out at designated locations in a Canadian forest and later retrieved and taken to the laboratory to rear the parasitoids out. These egg masses showed that the released parasitoids *Trichogramma minutum* dispersed 19 m in 5 days (Smith, 1988). The braconid parasitoid *Cotesia glomerata* was introduced into North America to control the caterpillar pest *Pieris rapae* in brassica fields, but there were fears that it might disperse into woodland and reduce populations of the native butterfly *Pieris virginiensis*. Benson et al. (2003) put out sentinel larvae of *Pieris rapae* and *Pieris napi* in woodland sites, and subsequent dissection revealed no *Cotesia* eggs or larvae in the sentinel hosts: they concluded that *C. glomerata* (even though present in adjacent meadows) do not forage in forested habitats and so are not a threat to *P. virginiensis*. To study dispersal of the parasitoid *Venturia canescens* in relation to environmental heterogeneity, Desouhant et al. (2003) released wasps (marked on the thorax with a dot of acrylic paint) from a central point and monitored their speed and pattern of spread out to 97 traps (moth larvae in food medium on 1 m high poles set 10 m apart). This design allowed the authors to relate dispersal to various factors such as sunlight intensity, amount and type of vegetation (Desouhant et al., 2003). Monitoring dispersal of mass-reared natural enemies of the same species as occur naturally in the area of release would be facilitated if the reared

individuals carry a genetic marker, such as an eye or body colour mutation (Akey, 1991), which is noticeable visually, or which can be detected unambiguously by biochemical means (Symondson & Liddell, 1996).

Some attempts have been made to obtain information on the movement of individuals without interrupting that movement. Baars (1979b) labelled adult carabids with an iridium isotope (^{192}Ir) by mixing it with quick-drying enamel paint. Using a portable scintillation detector, it was possible to track the movements of up to ten individuals over the same period of time in the field, although the beetles frequently lost their paint marks and there was some evidence from laboratory tests that the radiation affected beetle mortality rates.

The chemical composition of natural enemies (chemoprinting) might, potentially, be used to identify their area of origin, and thus be used to determine, indirectly, the minimum distance dispersed. This might be successful if they were to disperse as adults after pre-adult development on hosts/prey living on soils and vegetation with a characteristic chemical fingerprint. Wavelength dispersive X-ray fluorescence spectrometry can be used to make quantitative measurements of the elemental composition of individuals (Bowden et al., 1979). Dempster et al. (1986) used this method to measure the relative composition of nine chemical elements in adult Brimstone butterflies (*Gonepteryx rhamni*), and they found differences between sexes, sites, seasons and years. For this method to be of use, the chemical pattern obtained by pre-adult stages should be stable in the adult, and inter-site variation should be greater than intra-site variation. Unfortunately, in the Dempster et al. (1986) study, the differences gained in pre-adult stages were quickly masked as the adults aged and fed. Sherlock et al. (1986) also failed to demonstrate clear source-related chemoprints in the cereal aphid *Rhopalosiphum padi*.

The proportions and composition of stable isotopes, (e.g., deuterium), in the bodies of migrating animals may also reflect their area of origin (Hobson, 1999). Hobson et al. (1999) described the first application of the stable isotope technique to infer migration of an invertebrate. They showed that the stable hydrogen isotopic composition of wing keratin (metabolically inert after eclosion) of monarch butterflies (*Danaus plexippus*) was highly correlated with that in their milkweed host plants, which, in turn, was related to stable geographical patterns of deuterium in rainfall. Using these techniques it was possible to determine, approximately, the natal latitude in eastern USA of monarchs collected after a period of migration (e.g., when overwintering in Mexico) (Hobson et al., 1999). This technique has more recently been applied to studying hover fly (*Episyrphus balteatus*) migration in Western Europe (Raymond et al., 2014). Using a combination of wing morphometrics and hydrogen isotopic ratios, the authors concluded that hover fly populations in France were mostly made up of local individuals, with a limited amount of migration from more southerly populations. The chemical elements C, N, S, H and O all have more than one isotope and isotopic compositions can be measured with great precision using a mass spectrometer (Peterson & Fry, 1987). Stable C and N isotopes have been used in entomological studies. Carbon, for example, is fixed in C_3 plants (such as cotton) by the Calvin photosynthetic pathway and in C_4 plants (such as sorghum) by the Hatch–Slack pathway. C_3 and C_4 plants contain different ratios of isotopes ^{13}C and ^{12}C, and these ratios are maintained at higher trophic levels. Using a stable isotope mass spectrometer, Ostrom et al. (1997) showed that isotope values (isotopic ratios for C and N) were sensitive to change of laboratory diet by the ladybird *Harmonia variegata*, within a period of three weeks. These authors then documented diet shifts (alfalfa-, wheat- and maize-based feeding) in field-collected ladybirds (*Coleomegilla maculata*), which implied local dispersal of the beetles between habitats. Similar methods were developed by Ouyang et al. (2014) to study prey origins of the predatory beetle *Propylea japonica*. Hurd et al. (2015) used ^{13}C and ^{15}N ratios to demonstrate ontogenetic changes in the prey use of praying mantids, *Tenodera aridifolia sinensis*, by comparing stable isotope levels of field-collected

individuals to individuals fed known diets in the laboratory. Their results suggested that mantids do not feed on prey in the field in a frequency-dependent fashion, and that they prey upon species from higher trophic levels more commonly as they progress in their development.

Instead of relying on natural labelling (e.g., chemical fingerprint or stable isotope ratio), natural enemies can be labelled artificially with one or more specific trace elements (e.g., rubidium and strontium). This technique of elemental marking is defined as the establishment of an elemental concentration in an individual, significantly higher than that carried by the indigenous population (Akey, 1991). This method can be applied without having to handle the insect (as is necessary with paints, dyes, powders etc.), and it does not entail environmental contamination (as for radioactive isotopes). An atomic absorption spectrophotometer and graphite furnace permits analysis of micrograms of sample. Parasitoids and predators labelled with rubidium can be distinguished from controls for 4 to 20 days, depending on species. The method is useful for a wide range of parasitoids and predators (Graham et al., 1978; Prasifka et al., 2001). After plots of cotton and sorghum were treated with foliar solutions of rubidium chloride, a range of ladybirds, spiders, predatory beetles and predatory bugs became labelled and were found to have moved between fields. Recapture rates were three to four times higher than for the use of fluorescent dust markers in a comparable system (Prasifka et al., 1999, 2001). Elemental marking usually does not affect the biology of the labelled animal. One exception to this was a reduction in longevity of male parasitoids, *Anaphes iole* (Mymaridae) labelled with 1000 ppm rubidium. There were no adverse effects, however, on the parasitoid *Microplitis croceipes*, triple-labelled with caesium, rubidium and strontium at 1000 ppm (Jackson, 1991). Corbett et al. (1996) treated prune trees with rubidium in the autumn. Adult prune leafhoppers, *Edwardsiana prunicola*, became labelled and their mymarid parasitoid, *Anagrus epos*, carried a label 3.9 times greater than the background level of rubidium. Emerging parasitoids retained the label throughout their lifetime. Such labelling could, potentially, be used to quantify dispersal of the parasitoid from prune to grapevines, but, unfortunately, there was overlap in rubidium level between treated and natural populations of the parasitoid, and probabilistic statistical techniques will be needed to quantify dispersal in this context. Detection of marked eggs from marked mothers is possible, and so this technique can provide information on survival, dispersal and oviposition (Akey, 1991).

Small natural enemies may not be ideal candidates for marking with paints and tags, and detection of trace elements requires specialised equipment. An alternative is to mark by immersing the insects in rabbit immunoglobulin (IgG) and then assay (after dispersal) by ELISA (enzyme-linked immunosorbent assay) (Sect. 6.3.9) with goat anti-rabbit IgG. This marker can remain detectable for at least eight days. This is, however, an external marker, which will be lost on moulting or during development to a different life stage (Hagler et al., 1992a). Internal protein markers can be used for marking fluid-feeding natural enemies (Hagler & Jackson, 2001). Whitefly parasitoids (*Eretmocerus eremicus*) marked both internally (fed on honey solution containing rabbit IgG) and externally (exposed to a mist of rabbit IgG solution from a medical nebuliser), were released into a cotton field and 40% of recaptured individuals (during 32 h after release) were carrying the label. This study provided valuable information about gender-related dispersal, diel activity pattern, and distance moved by this parasitoid (Hagler et al., 2002).

Radio telemetry, a standard method for studying vertebrate dispersal, can also be used for studies of large invertebrate predators. Riecken and Raths (1996) fixed 0.7 g transmitters (with an aerial length of 5 cm and a battery life of 28 days) to the elytra of *Carabus coriaceus* (the largest species of carabid beetle in West Germany) using silicone glue. Heavy rain sometimes caused transmitter malfunction, but, despite such problems, the authors were able to detect beetles at a maximum range of 400 m. This method, unlike harmonic radar (below),

allows transmission at different frequencies, and, therefore, the individual tracking (without capture and disturbance) of more than one specimen in the same area (Riecken & Raths, 1996). This property of radio telemetry was exploited by Janowski-Bell and Horner (1999) to track individual tarantula spiders (*Aphonopelma hentzi*) in a Texas scrub habitat. Transmitters weighing 0.6–0.8 g were attached to adult male spiders weighing 2.5–7.5 g, and half of these spiders retained their transmitters for three or more days. The signal was received at a distance of several hundred metres and spiders could be detected in burrows and under rocks. Males were observed to move up to 1300 m during 18 days when searching for females. Although the possibility that the transmitter and aerial affected behaviour to some extent could not be excluded, tagged spiders were, nevertheless, observed to enter burrows and crawl past resident females, as normal (Janowski-Bell & Horner, 1999).

Remote sensing techniques, such as harmonic radar, can also be used to track the movement of natural enemies. The natural enemy target of harmonic radar tracking must carry a transponder (antenna plus diode), which detects radio waves transmitted from a hand-held device, and re-radiates some of the received energy at a harmonic frequency. This method does not require a battery to be attached to the target, which means that extremely lightweight tags can be developed (Kutsch, 1999). An harmonic radar system has been used to track the movements of large carabids over the ground (Lövei et al., 1997; Mascanzoni & Wallin, 1986; Wallin & Ekbom, 1988, 1994), and the foraging flights of bumblebees, *Bombus terrestris* (Osborne et al., 1999). Improved tags are now available for reflecting harmonic radar. The 0.4 mg tags are an order of magnitude lighter than those previously used on bees, and two orders of magnitude lighter than those used on carabids. Detection is possible from a distance of 50 m. The tag increased the weight of a tachinid fly by 0.1–0.9% and, 48 h after release, the fly had moved 100 m (Roland et al., 1996). In general, tags weighing up to 1.5% of insect body weight should have no deleterious effects on flight performance (Riley et al., 1996; Roland et al., 1996; Boiteau & Colpitts, 2001). Even lighter tags have now been developed for the study of colony collapse disorder in bees (Tsai et al., 2013). Metal detection equipment, employing a pulse field induction loop, is many times less expensive than harmonic radar. It is most suitable for detecting microhabitat preferences and local movement (e.g., to find pupation sites) of relatively sedentary cryptic invertebrates. Piper and Compton (2002) tagged beetle larvae with tiny steel tags (1 × 3 mm; 0.35 mg) and released them in leaf litter. Using a hand-held metal detector powered by a 12 V battery, they were able to detect 90% of the released larvae after five months in the field. The detection range is only 3–7 cm, but tagged animals can be detected through litter and soil, and laboratory experiments showed no significant effect of tagging on movement of larvae through leaf litter (Piper & Compton, 2002).

Other marking techniques which have been used to monitor the movements of natural enemies include: (a) numbered plastic discs glued to the dorsum of adult or larval carabids (Nelemans, 1986, 1988; Lys & Nentwig, 1991), (b) coloured and numbered bee tags attached to adult *Tenodera aridifolia* mantids (Eisenberg et al., 1992) and adult paper wasps (Schenk & Bacher, 2002), (c) spots of typewriter correction fluid applied to carabids (Wallin, 1986; Hamon et al., 1990; Holland & Smith, 1997), (d) spots of acrylic paint applied to the pronotum of adult eucoilid and braconid parasitoids with a fine brush (Driessen & Hemerik, 1992; Ellers et al., 1998), (e) numbers written on the wings of damselflies with a drawing pen (Conrad & Herman, 1990), (f) fluorescent powder applied to green lacewings (Duelli, 1980), and (g) stable isotopes fed to coccinellid adults in drinking water (Iperti & Buscarlet, 1986). Driessen and Hemerik (1992) attempted to mark adult *Leptopilina clavipes*, a figitid parasitoid of *Drosophila* flies, with micronised fluorescent dust. Although the dust worked well with the drosophilids, it was unsuccessful as a means of marking the parasitoids because it was rapidly lost from the smooth surface of the exoskeleton and elicited prolonged preening behaviour in the wasps. A potential problem with the use of dusts is that

they may become accidentally transferred to unmarked individuals of the same species during the recapture process. Fluorescent dusts have also been used to detect visits by parasitoids to specific plants by applying the dusts to the leaves of the plant and then searching for traces of the dust on parasitoids under UV illumination (Ledieu, 1977). Corbett and Rosenheim (1996) used parasitoid mark-recapture data to estimate the 'diffusion' parameter *D*, which is the random component of movement, independent of forces such as wind, attraction to resources and repulsion from conspecifics. Grape leaves containing leafhopper (*Erythroneura elegantula*) eggs, parasitised by the mymarid *Anagrus epos*, were put in a bag and gently rolled for 1 min in a mixture of 100 ml Day-Glo^{R} fluorescent powder and 1 kg walnut husks (as a carrier). As they emerged from the host egg, 85% of parasitoid adults marked themselves with a few minute particles of powder, which lasted at least 48 h, and permitted mark-recapture studies in vineyards (recaptures on yellow sticky traps). Marks were too small for detection in UV light, and a microscope was needed. Since only a few particles of marker adhered, minimal effects on survival and behaviour were expected (Corbett & Rosenheim, 1996).

Self-marking with fluorescent powder was also used for opiine braconid parasitoids of tephritid fruit flies in Hawaii in a study of dispersal in the field (Messing et al., 1993), but, unfortunately, this marker was found to greatly increase adult parasitoid mortality, compared to controls. Garcia-Salazar and Landis (1997) studied the effects of wet and dry Day-Glo^{R} fluorescent marker, at three doses, on the survival and flight behaviour of *Trichogramma brassicae*. In the dry method, powder was added to glass beads in a small plastic cup and shaken to build up a deposit on the walls. The beads and excess marker were removed and wasps were added and allowed to self-mark through contact with the walls. In the wet method, the marker was suspended in ethyl alcohol and swirled to coat the walls. Marking did not affect wasp survival, but the flight response was reduced at the highest dose for the dry method. More individuals, however, retained the mark for 24 h in the dry method. Adults of *Spalangia cameroni*, a hymenopteran parasitoid of the house fly *Musca domestica*, were allowed to self-mark in sawdust containing Day-Glo® fluorescent powder. Particles of fluorescent powder could be detected on the parasitoids with the naked eye for about four days, and marking reduced their survival only slightly, from eleven days to ten days (Skovgård, 2002).

Prasifka et al. (1999) marked predators with fluorescent dust in a sorghum field and made predator collections one day later in an adjacent cotton field. Examination of predators (mainly *Orius* bugs), under a dissecting microscope, revealed particles of dust, and demonstrated that predators had dispersed from the senescing sorghum crop into cotton. Six colours of fluorescent dust were used, with each colour being applied to a crop zone at a specific distance from the sorghum–cotton interface, so that the distance moved by predators could be determined. Dust was applied at 4.8 g per m of row at 3.5 kg cm^{-2} of pressure, through a compressed-air sandblast gun, with a spray nozzle internal diameter of 4.4 mm. Perry et al. (2017) developed a method to study the dispersal distances of a wide variety of forest-floor invertebrate species involving the deposition of three concentric rings of different-coloured fluorescent powder, and subsequent collection of invertebrates to record how many bands they had crossed (and thus the minimum distance they had travelled).

Thousands of individuals of the staphylinid beetle *Aleochara bilineata* were marked with DayGlo pigment and released weekly from 24 Canadian gardens. Three percent of marked beetles were recaptured with barrier pitfalls, water traps and interception traps in the gardens. Recaptured beetles had dye particles adhering to the intersegmental membranes of the abdomen, and at the bases of wings, head and coxae. Capture of marked beetles in control gardens (where no releases had been made) showed that they were capable of flying at least 5 km (Tomlin et al., 1992). Judging by experience with marking grasshoppers (Narisu et al., 1999), large natural enemies, such as carabid beetles, marked with fluorescent powder, should be detectable under

UV light at night, without the need for recapture, which would enable dispersal of the subject to be monitored without disturbance. Narisu et al. (1999) reported that 64% of marked grasshoppers were relocated with UV light at night, compared with only 28% by visual searching during the daytime. Schmitz and Suttle (2001) marked individuals of a large lycosid spider, *Hogna rabida*, with fluorescent powder and recorded (from a distance of 2.5 m using binoculars) horizontal and vertical distances moved every five minutes until the spider noticed the presence of an observer (which could take up to 6 h).

Natural enemies with pubescent cuticles could be marked with pollen of a species that does not occur in the study area. Many pollens can be identified to species with a microscope, they are durable and some have evolved to adhere tenaciously to the insect exoskeleton. In some contexts, ingested pollen can also yield valuable information about natural enemy dispersal. Wratten et al. (2003) dissected the guts of adult hover flies, stained the contents with saffranin (White et al., 1995), and were able to detect pollen of *Phacelia tanacetifolia* which the hover flies had taken from strips of this flower planted at the edge of fields. This method was exploited to quantify the degree of inhibition of hover fly dispersal attributable to various types of hedges and fences in the farmed landscape. *Phacelia* pollen was detectable in the hover fly gut for less than 8 h, which enabled calculation of a crude estimate of rate of dispersal (Wratten et al., 2003).

Vital dyes could prove useful in dispersal studies. Braconids were labelled with the vital dye acridine orange, through their food. The alimentary canal and eggs of these parasitoids became labelled within 24 h, and could be seen fluorescing under an epifluorescent microscope. Tissues continued to fluoresce for 6 weeks after death. Parasitoid longevity was unaffected, and no behavioural effects were observed. Oviposited eggs of some species were labelled, and the label persisted for about 2 days. Stained eggs hatched normally and eventually produced adult offspring (Strand et al., 1990).

Topham and Beardsley (1975) labelled tachinid parasitoids of sugarcane weevils with a radioactive marker, in an attempt to monitor the distance travelled by the adult flies between oviposition sites within crops and nectar food sources at field margins (Sect. 6.5). Adults of the eulophid wasp *Colpoclypeus florus*, a parasitoid of leafrollers, were fed on honey-water containing the radioisotope ^{65}Zn. Labelling did not affect fecundity, but it significantly reduced longevity and the viability of oviposited eggs (Soenjaro, 1979). Emerging adults of the egg parasitoid *Trichogramma semifumatum* were fed on ^{32}P honey-water solution. Three-and-a-half million wasps were released in Californian alfalfa and sampled with sweep nets and vacuum insect nets. Only a few hundred were recaptured, due to rapid dispersal and dilution of marked wasps in the area, but tagged wasps lived at least 15 days after release, and the study provided useful data on rates of dispersal (Stern et al., 1965). *Trichogramma ostriniae* (an egg parasitoid of the Asian corn borer, *Ostrinia furnacalis*) were dipped or sprayed with ^{32}P, because less than 25% became marked if allowed to feed on radioactive honey-water. The label did not affect longevity or reproduction, but 63–83% of the label was lost over five days in the field, probably due to self-grooming. The method has potential for short-term ecological studies in situations where radioactive markers are permitted (Chen & Chang, 1991). In some countries there are environmental concerns and regulations concerning the use of radioactive markers in field studies (Greenstone, 1999). Southwood and Henderson (2000) provide further information on marking techniques and the use of codes, which allow the recognition of large numbers of individuals.

In the context of commercial rear-and-release biocontrol programmes, quantitative assessments of factors that affect searching efficiency (such as flight initiation and walking activity) of parasitoids is an important aspect of quality control (Bigler et al., 1997). A simple laboratory test was devised and showed that strains of *Trichogramma brassicae* varied considerably in flight initiation (percentage flyers and non-flyers in a three day post-emergence period at 25 °C). Results from field cage studies, using cages with clear plastic walls and sticky strips, confirmed the laboratory results (Dutton & Bigler, 1995). The laboratory

flight-test apparatus used by Ilovai and van Lenteren (1997) to monitor quality of mass-reared parasitoids (*Aphidius colemani*) consisted of a glass cylinder with the internal wall coated with repellent, and the exterior wall shaded to exclude light. Mummies were placed at the bottom of the cylinder and emerging parasitoids flew up to the light at the top of the cylinder, where they were caught on a glass plate, coated with adhesive. Only 40–65% of emerging adults were found to be capable of flight.

The technology is available to study patterns of diel activity efficiently in the laboratory, with a combination of robotics and video image analysis, in real time. A video camera can be moved, automatically, to partition its time between a number of subjects. A system such as this was used to analyse diel cycles of locomotor activity by *Trichogramma brassicae*, measuring the percentage of time spent moving, linear speed, angular speed, and sinuosity of trajectory. Forty individuals were studied, with one 5 s recording every 5 min (Allemand et al., 1994). For most parasitoids, the last stage of host-finding is by walking, and parasitisation rate is heavily influenced by rate of walking and searching. With this in mind, Wajnberg and Colazza (1998) video-recorded the walking path of *T. brassicae*, and used an automatic tracking computer device to transform the path to X–Y coordinates (with an accuracy of 25 points s^{-1}). The track width was set at two reactive distances (this species can perceive host eggs at a mean reactive distance of 3.7 mm), and the area searched per unit time was computed with each surface unit counted once, even if the wasp crossed its own path. *T. brassicae* was found to search, on average, at 28 mm^2 s^{-1}. The method can be used to guide genetic selection of mass-reared *Trichogramma* species. Using similar technology, Van Hezewijk et al. (2000) found a significant amount of variation between strains of *Trichogramma minutum*, and within strains over time. A wide variety of automated video-tracking systems are now available to record insect behaviour, some of which are low-cost and open source (reviewed in Dell et al., 2014). In addition, automated locomotor activity monitors that record insect activity by recording numbers of infrared beam breaks inside replicated, isolated units (e.g., glass tubes) are commercially available and easy to use (Pfeiffenberger et al., 2010). To date, these set-ups have mostly been applied to neurological and genetic studies of *Drosophila*, but they could also be usefully applied to studying circadian rhythms and relative activity levels of different species or intraspecific variants of natural enemies in the laboratory. Rasmussen et al. (2018) conducted one of the first such studies applied to a biological control agent.

DNA-based techniques (Sect. 6.3.12) may enable dispersal of natural enemies released in biocontrol programmes to be distinguished from that of native natural enemies of the same species, and also permit tracking of the genetic marker through successive generations (which would not be possible with other marker systems) (Greenstone, 2006). Gozlan et al. (1997) attempted to use RAPD-PCR (Sect. 6.3.12) to distinguish between strains of three species of *Orius* (predatory heteropteran bugs) used in augmentative release programmes. Species were readily distinguished, but a high degree of polymorphism prevented discrimination between strains. This method was used successfully, however, by Edwards and Hoy (1995), to monitor insecticide-resistant biotypes of *Trioxys pallidus* (a parasitoid of the walnut aphid, *Chromaphis juglandicola*) released into orchards, and to distinguish them from wild populations. The resistant biotype disappeared from one orchard by a year after release, but it was detected for three years in two other orchards. It might be possible to use PCR techniques to monitor dispersal of virus-labelled natural enemies. De Moraes et al. (1998) successfully used PCR to detect nucleopolyhedroviruses in homogenates of hemipteran predators up to 48 days after application of these viruses in a soybean field. Although peak levels of virus detection occurred at 10–45 days after application, the virus was found in predator homogenates five days before it was found in the host larvae (velvetbean caterpillar, *Anticarsia gemmatalis*), suggesting that the foraging predators became externally contaminated with virus particles.

Similarly, Smith et al. (2000) used PCR to detect genetically engineered baculoviruses in predators (spiders, ladybirds and heteropterans) five days after a cotton field was sprayed with these viruses. If natural enemies, before release, were deliberately surface-contaminated with a unique (e.g., genetically manipulated) virus, this could be useful for short-term dispersal studies (baculoviruses are usually inactivated by UV light after two or three days).

6.3 Determining Trophic Relationships

6.3.1 Introduction

In nature, the trophic interrelationships of invertebrates within a community rarely, if ever, consist of simple food chains (Sect. 6.1). They often comprise an extensive feeding web composed of several trophic levels. The comparative ease with which parasitoids of endophytic hosts can be reared from, and their remains (egg chorions, larval exuviae) located within, structures such as leaf mines, stem borings and galls, together with the consequent certainty with which parasitoid–host relationships can be discerned, has meant that some of the most detailed studies of food webs involving insects have been on gall-formers, leafminers and stem borers (Askew & Shaw, 1986; Redfern & Askew, 1992; Claridge & Dawah, 1993; Memmott et al., 1994; Dawah et al., 1995; Martinez et al, 1999; Maunsell et al., 2015). Food webs associated with endophytic insects are also attractive to researchers because of their greater complexity. Askew (1984) has documented more than 50 species of cynipid gall wasps, and their associated parasitoids, on oak and rose galls in Britain, and Redfern and Askew (1992), Claridge and Dawah (1993), Valladares and Salvo (1999), Memmott et al. (1994), Dawah et al. (1995) Lewis et al. (2002), Maunsell et al. (2015) give diagrammatic representations of a range of food webs based on gall-formers, stem borers and leafminers. Complex webs have also been constructed for non-endophytic pests such as aphids, their parasitoids and secondary parasitoids (Müller et al., 1999; Lohaus et al., 2013) as well as for lepidopterans (Henneman & Memmott, 2002; Timms et al., 2012; Peralta et al., 2014; Wirta et al., 2014; Rocca & Greco, 2015; Frost et al., 2016; Shameer et al., 2018;). They have also been used to answer specific ecological questions, such as the degree to which parasitoid biocontrol agents are penetrating natural communities (Henneman & Memmott, 2002), the impact of the establishment of introduced herbivores on native food webs (Timms et al., 2012), the importance of functional complimentarity and redundancy of parasitoids as determinants of parasitism rates (Peralta et al., 2014), and to quantify the importance of apparent competition in trophic webs (Frost et al., 2016). Further considerations on food webs, their construction and use can be found in Sect. 6.3.12.

Described below are several methods used (Table 6.3 summarises their relative merits) for elucidating the trophic relationships between invertebrates and their natural enemies from the standpoint of: (a) the host or prey, i.e., the species composition of its natural enemy complex and (b) the natural enemy, i.e., its host or prey range. Some of these methods may also be used when the trophic relationships are already known, either to confirm that a relationship exists in a particular locality, and/or to record the amount of predation and parasitism: their use in quantitative studies is largely dealt with in Chap. 7. In cases where a predator is likely to be feeding in more than one ecosystem during a relatively short period of time, analyses using stable isotopes of carbon and nitrogen (Fantle et al., 1999; Post et al., 2000; Post, 2002) might be applicable for determining the relative contribution of prey from each ecosystem to the overall diet of the predator (but without any precision concerning exactly which species have been consumed). Stable isotope analyses applied to body tissues can summarise some general aspects of the diet of an individual over a long period of time, as compared with the various methods of gut contents analysis (Sects. 6.3.7–6.3.12) which describe foods consumed during one or a few meals. Stable isotope ratios

Table 6.3 Comparison of methods for investigating qualitative aspects of trophic relationships in natural enemy complexes and communities. Advantages and disadvantages of each method are discussed in more detail in the text (Sect. 6.3)

Method	Advantages	Disadvantages
Field observation	Immediate and usually unequivocal results obtained with minimal equipment	Time-consuming and labour-intensive; small, hidden, nocturnal, subterranean or fast-moving invertebrates difficult to observe; possibility of disturbance to invertebrates during observation; high cost of video equipment; unlikely to observe predation by highly mobile predators at natural (low) densities
Collection of prey and remains from within and around burrows and webs	Prey items conveniently located in or around nest/burrow/web	Often involves disturbing predators; applicable to few natural enemies; invertebrates caught in webs may not be prey
Tests of prey/host acceptability	Results usually unequivocal Defines prey/host that a predator/parasitoid is physically and behaviourally *capable* of attacking	Identification of parasitoid immatures to species level often not possible due to lack of descriptions and keys; false negatives a problem; may not apply to prey choices in the field (false positives)
Rearing parasitoids from hosts	Results usually unequivocal	False negatives a problem, if sampling is not sufficiently extensive and intensive; hosts of koinobionts need to be maintained until parasitoid larval development is complete
Gut dissection and faecal analysis	Simple and quick to perform with minimal equipment; samples can be stored either frozen or in preservative for long periods prior to testing; direct information on prey consumption in the field	Can only be used if predator or parasitoid ingests solid, identifiable parts of prey or hosts that possess solid parts; identification of prey remains not always possible; possibility of misleading results due to scavenging and secondary predation
Immunological analyses of gut contents	Accurate, highly sensitive and reproducible techniques available; cell lines generating monoclonal antibodies can be retained for future use, giving reproducibility to assays over time; sample can be stored frozen for long periods prior to testing; large numbers of predators can be analysed rapidly	Time-consuming to develop and calibrate, requires specialist equipment and expertise; risk of false positives through cross-reactions, scavenging (and, possibly, secondary predation); has been superseded by DNA-based techniques
Prey labelling	Simpler to perform than either electrophoretic or immunological tests; dyes easily detected; samples can be stored frozen for long periods prior to testing	Expensive equipment required to detect some labels; radioactive labels a hazard to operator and environment; usually involves severe disturbance to the system under study; labels can be lost rapidly; label passes through trophic levels by a variety of routes (e.g., coprophagy)

(continued)

Table 6.3 (continued)

Method	Advantages	Disadvantages
Electrophoresis	Can be accurate and sensitive once standardised; large numbers of samples can be analysed rapidly	Expensive, time-consuming to calibrate, and requires specialist equipment and expertise; risk of false positives through scavenging and secondary predation; inappropriate for highly polyphagous species (coincidence of diagnostic bands); has been superseded by DNA-based techniques
DNA-based techniques	Can provide highly accurate data identifying predation on different species, biotypes and populations; all prey in the predators can be identified simultaneously using high-throughput sequencing	Requires molecular biology facilities; risk of false positives through scavenging, secondary predation and (where PCR is involved) cross-contamination; some species detected by high-throughput sequencing may not be on sequence databases (may need to create your own sequence database)

(Sect. 6.2.11) are useful natural tracers of nutrient flows in ecosystems (Lajtha & Michener, 1994; Ponsard & Arditi, 2000) and could be used to provide a broad summary of how organic matter flows through a food web (Sect. 6.3.12), including cryptic food webs in soil (Eggers & Jones, 2000; Scheu & Falca, 2000). $^{15}N/^{14}N$ ratios, for example, tend to increase with each successive link in a food chain (e.g., plant–herbivore–predator) because deamination and transamination enzymes preferentially remove light (^{14}N) amine groups and the $^{15}N/^{14}N$ ratio in organisms becomes progressively heavier than that of atmospheric nitrogen. Excreted nitrogen is, therefore, lighter than body tissue nitrogen. Interpreting the isotopic signature of a consumer in terms of its trophic position needs to be in relation to an appropriate isotopic baseline. Post (2002) discusses methods for generating an isotopic baseline and evaluates assumptions underlying estimation of trophic position. In the northern temperate lake ecosystems studied by Post (2002), snails were a suitable baseline for the littoral food web and mussels for the pelagic food web. Lycosid spiders, *Pardosa lugubris*, were shown to have higher $^{15}N/^{14}N$ ratios than their prey, suggesting that stable isotope analysis may be appropriate for assessing the trophic position of the organisms tested (Oelbermann & Scheu, 2002). ^{15}N content in predators, however, is affected by food quality and starvation (Oelbermann & Scheu, 2002). Such effects need to be taken into account in determining trophic position. In addition, scavenger species often have ^{15}N levels within the range of values found for predators (Ponsard & Arditi, 2000), which needs to be acknowledged when interpreting results. Stable isotope ratios have been used to elucidate features of rocky littoral food webs that were not detected by analysis of gut contents (Pinnegar & Polunin, 2000). Using stable isotope analysis of carbon and nitrogen, McNabb et al. (2001) found that linyphiid and lycosid spiders in experimental cucurbit gardens treated with straw and manure mulches appeared to be preying largely on detritivores (such as Collembola). They caution, however, that ^{13}C values of Collembola were not significantly different from those for spotted cucumber beetle (*Diabrotica undecimpunctata howardi*), which was a major herbivore in the system under study. Thus, in some cases, stable isotope analysis may lack the precision to discriminate between consumption of detritivores and herbivores. A very useful review of the topic summarising the methodology involved, and highlighting the usefulness of the method for understanding predator–prey dynamics in the field, is available elsewhere (Hood-Nowotny & Knols, 2007).

Unbiased sampling of predators that are to be assayed by post-mortem methods (e.g., gut dissection, immunoassays, electrophoresis, DNA

techniques) should, ideally, employ at least one sampling method relying on predator activity (e.g., sticky trap, Malaise trap, pitfall), run for at least 24 h (to accommodate the predator's diel activity cycle), and one that will include inactive predators (e.g., ground search). The latter is not, however, always feasible; for example, with large carabids that are frequently found at densities well below one per square metre, in which case data should be interpreted with caution. Violent methods (e.g., sweeping, beating, suction sampling) are not advisable in circumstances where damaged target prey could contaminate the predators and produce false positives (Crook & Sunderland, 1984). Dry pitfall traps should be avoided if there is a significant degree of entry of the traps by the target prey (i.e., if the density of prey in the traps is greater than that found in the same area of open ground), because artificial confinement of prey with hungry predators may produce an erroneous assessment of prey consumption in the field. This can sometimes be overcome by using dry traps with a mesh insert, so that larger predators are separated physically from smaller prey. There is evidence that this works well for negatively phototactic prey, such as earthworms, which remain in the bottom of the trap, but less well for positively phototactic prey, such as aphids, which can climb up the sides of the trap, and thus come within reach of the predators once more (W. O. C. Symondson, D. M. Glen, M. L. Erickson, J. E. Liddell and C. Langdon, unpublished data). An additional problem can be predation by one predator upon another within the trap, where the predator consumed has previously fed on the target prey. Hengeveld (1980), for example, found the incidence of spider remains in the guts of carabid beetles to be much greater when the beetles were collected in live-trapping pitfalls than in funnel traps where the beetles were killed instantly in 4% formalin solution. The inclusion of stones, soil or mesh can help to minimise this problem in dry traps. If the predator of interest is, for example, a large carabid, then holes in the bottom of the trap can allow small predators, and other target or non-target prey, to escape. Wet traps can also lead to biased results. Water can attract prey, such as slugs (and possibly many insects during dry weather), to the local area of the trap leading to increased consumption by predators in that area (Symondson et al., 1996). It has also been shown that when slugs drown in a trap, carabid beetles drowning in that same water may ingest proteins released by the dying or dead prey, again leading to false positives (W. O. C. Symondson, unpublished data). Trapping reagents that cause protein denaturation (e.g., formalin, alcohol) should be avoided. Regular emptying of traps, and transport of predators on ice, will reduce the rate of microbial degradation of the gut contents of dead predators (Sunderland, 1988), and slow down digestion in live predators caught in dry traps (Symondson et al., 1996).

With most of the post-mortem methods described below, it is advisable to carry out some initial laboratory studies with all predator species that will be collected from the field for testing. Predators that have just eaten a large meal of the target prey should be tested. This initial screening will identify any species that have an extreme degree of extra-oral digestion (Cohen, 1995), and which will therefore often be negative in the test, even though they may be important consumers of the target prey in the field. The rates at which prey remains decay within predators are discussed in Sect. 6.3.9.

False negatives are a problem common to all the methods; that is, due to undersampling, some parasitoid or predator species comprising an insect's natural enemy complex may be overlooked, as may certain insect species comprising a natural enemy's prey/host range. More serious, however, is the problem of false positives with several of the methods discussed here (e.g., tests of prey and host acceptability, immunoassays, prey/host labelling, electrophoresis). Positives in post-mortem tests indicate consumption of the target prey, but not necessarily predation, because the label can enter the predator by other routes, such as scavenging and secondary predation (Sunderland, 1996), or by consumption of autotomised parts of the prey (e.g., the slug *Deroceras reticulatum* sheds the posterior part of its foot and escapes when attacked by a large generalist carabid beetle, which then eats the autotomised

organ—Pakarinen, 1994), or by trophallaxis (Morris et al., 2002). An investigation of an aphid–spider–carabid system suggested that, at least in this system, secondary predation will cause little error in predation estimates; detection of secondary predation using a sensitive anti-aphid monoclonal antibody (Sect. 6.3.9) only occurred where carabids were killed immediately after consuming at least two spiders, which were, in turn, eaten immediately after consuming aphids (Harwood et al., 2001b). Under certain circumstances there may be no requirement to separate predation from scavenging. In the study by Symondson et al. (2000), monoclonal antibodies were used to assess the importance of earthworms simply as an alternative source of non-pest food that could help maintain populations of the carabid *Pterostichus melanarius* when pest density was low. In this scenario, dead earthworms were as useful as live ones.

6.3.2 Field Observations on Foraging Natural Enemies

Watching natural enemies in action is the simplest and most unequivocal for gathering evidence on the trophic relationships within both predator–prey and parasitoid–host systems. Generally, it falls into two areas. First, direct observation with the naked eye and binoculars in the field (Holmes, 1984; Heimpel & Casas, 2008), and second, remote observation using video-recording apparatus. There are, however, some practical drawbacks to these techniques: they are often very labour-intensive, data often accumulate very slowly, and data may be of limited value if the trophic relationship itself is disturbed during observations. In addition, many natural enemies are nocturnal, live concealed in the soil, or are very small, creating difficulties for observation. Sometimes, predator–prey interactions under a closed crop canopy within arable ecosystems are the focus of study, making observation without disturbance particularly difficult. Such drawbacks are especially important when studying minute, but often very fast-moving, parasitoids. An added complication when attempting to record oviposition by endoparasitoids is that it is sometimes difficult to assess whether or not an ovipositor insertion has in fact resulted in an egg being laid (Chap. 1).

Despite the drawbacks outlined above, direct field observation has been used in a number of studies of predation and parasitism. Workers have either used prey that has deliberately been placed out in a habitat (sentinel prey), or they have used naturally occurring populations of prey. Greenstone (1999) tabulates studies in which direct observation has been used to determine the spectrum of spider species attacking pests.

Predation of the eggs of cotton bollworms, *Helicoverpa zea*, was investigated by attaching batches of eggs to the upper surface of cotton leaves, using fresh egg white and a small paint brush (Whitcomb & Bell, 1964). The eggs were observed directly for 12 h periods during daylight and all acts of predation were recorded. Predators were identified whenever possible, and the type of feeding damage inflicted was noted. Ants tended to remove eggs completely, explaining the disappearance of eggs from cotton plants recorded in earlier experiments. Using a similar approach, Buschman et al. (1977) allowed adults of the velvetbean caterpillar, *Anticarsia gemmatalis*, to lay eggs on soybean plants in laboratory cages, and then placed the egg-laden plants out in soybean fields. The plants were continuously observed through the daylight hours by a team of observers working in 2 h shifts. A critique of the use of sentinel prey as a technique has been published recently (Lövei & Ferrante, 2017).

Weseloh (1989) identified potential ant predators of spongy moth larvae by placing moth larvae on the forest floor in an area where a particular ant species was seen to be foraging, and then noting the ants' behavioural reaction to the larvae. Morris et al. (1998), in their study of predation by ants (*Tapionoma nigerriumus*) on the olive moth, *Prays oleae*, recorded the number and proportion of ants carrying this prey, as they approached the nest along one of their many trails, during 10 min intervals throughout the day. Interestingly, far fewer ants were seen carrying *P. oleae* than might have been suggested

by analyses using ELISA (Morris et al., 1999b, 2002). This was explained by the fact that ants participate in trophallaxis, the exchange of food between individuals, and illustrates very well the need to complement biochemical approaches with direct observation and/or behavioural studies. Riddick and Mills (1994) used binoculars, and a red flashlight, to observe carabid predation of tethered codling moth (*Cydia pomonella*) larvae in an apple orchard. Red-light illumination is used to minimise disturbance to the predator and prey but allow observation. Binoculars enabled the researchers to monitor the larvae from several metres away and allowed identification of the carabids, but without disturbing their behaviour. Similarly, Villemant and Ramzi (1995) used binoculars to observe predation on egg masses of *Lymantria dispar* on cork oak. A large wolf spider, *Hogna rabida*, was the focus of observation by Schmitz and Suttle (2001), but this species was found to retreat into the litter if researchers approached within 1.5 m. To circumvent this problem, individual spiders were dusted with fluorescent powder and observed from 2.5 m using binoculars.

Diurnal predation of the cotton fleahopper, *Pseudatomoscelis seriatus* (Heteroptera, Miridae), was observed by walking through cotton crops (Nyffeler et al., 1992) (Sect. 6.2.6). During 1 h observation periods, the number of predators without prey, the number of predators with fleahopper prey, and the number of predators with alternative prey were all counted. Predators carrying prey were collected, and both the predator and its prey were identified later in the laboratory. Nyffeler (1999) collated data from 31 similar published observational studies of spiders attacking pests in the field. Lavigne (1992) observed the predatory behaviour of Australian robber flies (Diptera, Asilidae) in pastures, using two main approaches: continuous observation of single flies over extended periods, and transect walks to record as much behaviour as possible. Feeding flies were collected, identified, and finally released, once the prey had been removed for identification. Similarly, Halaj and Cady (2000) made 30–60 min walks from 0800 to 1100 and 2130 to 0030 (using headlamps), and recorded feeding activity by harvestmen (Opiliones) in a soybean field and an adjacent hedgerow. When feeding was observed, the harvestman and its prey were preserved in alcohol for later identification in the laboratory. Van den Berg et al. (1997) made daytime visual observations of predator feeding rate on soybean aphids during 10 min periods. Their data supported calculation of functional responses for a ladybird, *Harmonia arcuata*, and a rove beetle, *Paederus fuscipes*, but the foraging of spiders, ants and crickets was found to be too cryptic for application of this method.

Holmes (1984) examined individual colonies of the cereal aphid *Sitobion avenae* on selected ears of wheat, identified by a plastic label, and re-examined them at regular intervals, to observe the searching behaviour of staphylinid beetles. The data not only confirmed that aphids were consumed, but also provided quantitative information on the rate of prey consumption by predators.

Griffiths et al. (1985) studied nocturnal carabids in cereals, using arenas placed out in the field at night, and illuminated with red light, to which the carabids do not respond. It is, however, important to note that not all arthropods are insensitive to red light (Sunderland, 1988): harvestmen (Opiliones) and springtails (Collembola) are known exceptions. Many such studies do not yield quantitative data, since the prey has to be removed to be identified, and the predator's normal behaviour is disturbed (Chap. 7 discusses quantitative aspects of this technique).

Diel activity patterns of a squash bug egg parasitoid, the scelionid *Gryon pennsylvanicum*, were recorded at 3 h intervals in a squash field. A range of behaviours, which included 'probing-ovipositing', was recorded by direct observation. A red flashlight was used at night (Vogt & Nechols, 1991). Landis and van der Werf (1997) observed sugar beet plants during the daytime for 200 min per day, and took care not to touch the plants, or cast shadows on them. Aphids were observed being eaten on six occasions, including by cantharid and anthicid beetles.

Rosenheim et al. (1993), using field enclosures, showed that predation by heteropteran bugs on lacewing larvae (intraguild predation)

can release cotton aphids from effective biological control. Because of the possibility that enclosures might distort the normal behaviour of the predators in this system, Rosenheim et al. (1999) later made 450 h of direct visual observations in cotton fields, and demonstrated that lacewing larvae (*Chrysoperla carnea*) were, indeed, killed frequently by predatory Heteroptera (various species of *Orius*, *Geocoris*, *Nabis* and *Zelus*).

A few studies have managed to directly observe behaviour of several species of parasitoids in the field, even very minute species (Heimpel & Casas, 2008). For example, Heimpel et al. (1996) were able to confirm an association between the likelihood for *Aphytis* spp. parasitoids to host feed versus oviposit and their egg load by directly following individuals in the field and observing their behaviour. Similarly, one of the only studies to directly quantify predation on parasitoids was conducted by Heimpel et al. (1997), who conducted almost 90 h of direct observation of foraging or resting parasitoids and determined that spiders, ants, and assassin bugs preyed on *Aphytis* spp. on almond trees.

With sufficient ingenuity, even predation occurring below the soil surface can be monitored directly. Brust (1991) observed underground predation of larvae of southern corn rootworm (*Diabrotica undecimpunctata*) on maize roots. He positioned a Plexiglass plate close to the roots, and had a bag of soil that could be removed to allow root observation at 2 h intervals for 24 h. This method showed that carabid larvae (in the genera *Harpalus* and *Pterostichus*) were significant predators of first- to third-instar rootworm larvae. A similar method was used to investigate predation of eggs of southern masked chafer (*Cyclocephala lurida*) and Japanese beetle (*Popillia japonica*) by ants in turfgrass (Zenger & Gibb, 2001). Such approaches are excellent for observation of predatory behaviour and for confirming trophic links but provide limited quantitative information.

Time-lapse video-recording techniques, which reduce the arduous and time-consuming nature of direct observation, are available (Wratten, 1994). These techniques are very useful for observing attacks on relatively sedentary prey, but are not appropriate, in most cases, for recording attacks on small active prey, or for monitoring the foraging behaviour of highly mobile natural enemies in complex three-dimensional habitats, such as plant canopies. Recording is possible at very low light intensities, or under infrared light, thus reducing disturbance (Howling & Port, 1989; Howling, 1991; Schenk & Bacher, 2002). Amalin et al. (2001), using a video time-lapse cassette recorder and red-light illumination (5.0 lx) in the laboratory, discovered that nocturnal clubionid spiders are able to detect leafminers through the leaf epidermis and then either puncture the mine and eat the larva in situ, or cut a slit in the mine and remove the prey. Laboratory studies of the foraging behaviour of some slug species have been carried out by several workers (Bailey, 1989; Howling & Port, 1989; Howling, 1991) and the methods could be adapted for studying the foraging behaviour of predators. Halsall and Wratten (1988b) used time-lapse video equipment to compare the responses of polyphagous predators to plots of high and low aphid density in a winter wheat field. Cameras were focussed on a 9 cm × 11 cm patch of moistened silver sand within each plot, and night illumination was provided by 100 W IR lights, powered from a portable generator. The nocturnal carabid, *Anchomenus dorsalis* (= *Agonum dorsale*), was found to make a greater number of entries into the high than the low aphid density plot. Similar methods were used in a field of winter wheat by Lys (1995), who focussed the camera on a 10 cm × 10 cm area of ground where *Drosophila* puparia stuck to cardboard had been placed. In addition to securing data on relative predation rates by diurnal and nocturnal predators, this study provided fascinating insights into the agonistic behaviour of carabids under field conditions (e.g., *Poecilus* (= *Pterostichus*) *cupreus* attacked and repelled conspecifics as well as smaller predators). The simultaneous use of multiple video cameras in the field can enable a more efficient collection of quantitative data on predation and parasitism in some systems. Meyhöfer (2001) used a system of 16 cameras

equipped with infrared diodes for night vision, a video multiplexer and a time-lapse video recorder to study predation of aphid mummies in a sugar beet field. Camera input routed via the multiplexer was stored on a single videotape (720 h of observation on a 3 h tape). Schenk and Bacher (2002) employed a similar range of equipment to study predation on sedentary late-instar larvae of the shield beetle, *Cassida rubiginosa*, on creeping thistle. Equipment was checked several times daily, and, when necessary, cameras were re-focussed or missing larvae replaced. Examination of the video tapes showed that 99% of predation events were due to the paper wasp, *Polistes dominulus*. Technological advances now allow the input of up to 72 cameras to be stored directly to computer hard disc (Meyhöfer, 2001). Security and protection of video equipment is an important consideration in field studies. Practical advice concerning equipment, arenas and automatic analysis can be found in Varley et al. (1993) and Dell et al. (2014).

The advent of digital recording has greatly improved the ability of researchers to remotely observe predators in action. Digital technology is cheaper, has higher video resolution and is easier to deploy. The results obtained using these techniques can often be surprising and at odds with the predators caught using more traditional methods such as pitfall traps. For example, Grieshop et al. (2012) found that, in three different agroecosystems, ants were the most important predators and beetles were much less effective, despite their abundance in pitfall traps. In addition to being less time-consuming, digital surveillance of predators can be used to compare different sampling methods. For example, a study comparing predators of *Aphis glycines*, using vacuum sampling, direct human observation and digital surveillance, showed that the former method was better at assessing abundance, but that video surveillance, although missing some of the smaller predators, gave a better estimate of time spent foraging (Wolz & Landis, 2014). As mentioned earlier, sentinel prey are often used to estimate predator and parasitoid abundance and effectiveness. Whilst this may be suitable for normally immobile prey items such as eggs and pupae, the use of immobilised, normally active prey items may skew results. Digital video surveillance of live and sentinel Brown Plant Hopper in the field showed that frogs were the major predators of live individuals, whereas sentinel leaf hoppers were mainly taken by spiders and water bugs (Zou et al., 2017).

Because an individual act of predation is often completed in a relatively short period of time, and predators spend only part of their time foraging for food, the number of records of predatory acts occurring during an observation period can be very low (Sterling, 1989). Consequently, the likelihood of observing any acts of predation is often low for many predator species. It is sometimes possible to increase the rate of field data accumulation by artificially increasing prey densities. In an investigation of spider predation on the rice green leafhopper, *Nephotettix cincticeps*, Kiritani et al. (1972) placed, in paddies, rice plants that had been artificially infested with unnaturally high numbers of leafhopper eggs. Observers patrolled the rice plots after the eggs had begun to hatch, and recorded occurrences of spiders feeding on leafhoppers, noting the species of both the predator and the prey involved. Similarly, Way et al. (2002) planted potted rice plants infested with the brown planthopper, *Nilaparvata lugens*, into tropical upland rice fields and observed predation by ants both on the plants and on the ground below. Direct observation can be used to estimate predation rates (Greenstone, 1999), if data are collected, not only on the percentage of predators seen with prey, but also on the handling time (the period from attack to cessation of feeding), and on the time available for prey capture in the field (Nyffeler & Benz, 1988b).

6.3.3 Collection or Examination of Prey and Prey Remains

If the predator does not consume all of its prey, the remains of the cadaver may display distinctive marks that enable the perpetrator to be identified. For example, Neuroptera leave a pair of small holes, Heteroptera a single hole, and

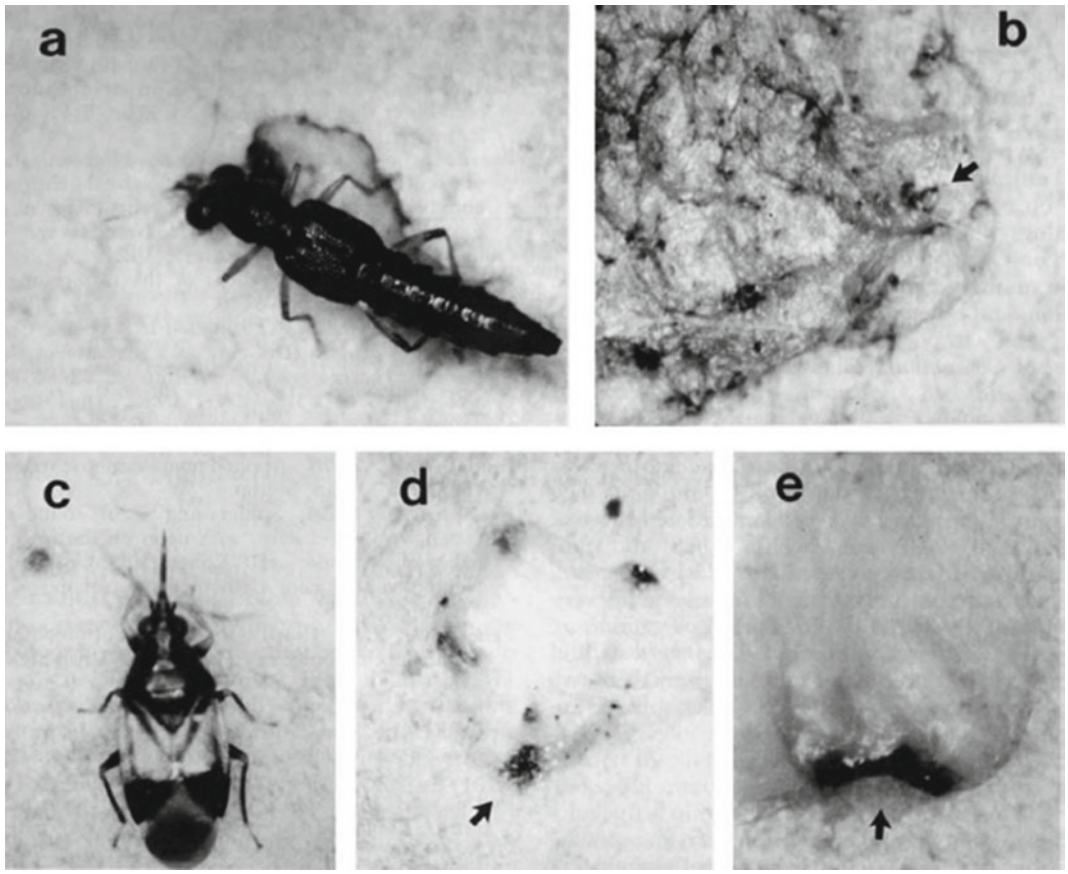

Fig. 6.23 Damage caused to egg masses of *Ostrinia nubilalis* (Lepidoptera: Pyralidae) by various predators: **a** predation by the staphylinid beetle *Stenus flavicornis*, **b** predation by a lacewing larva (*Chrysopa* sp.), arrow marks a feeding hole, **c** predation by the heteropteran bug *Orius insidiosus*, **d** damage from predation by *O. insidiosus*, arrow marks fungal growth at feeding hole, **e** damage from mite predation, arrow marks damage. *Source* Andow (1990). Reproduced by permission of The Entomological Society of America

ants a peppering of small holes (Mills, 1997). Andow (1990) studied, in the laboratory, the nature of the damage caused to egg masses of *Ostrinia nubilalis* (Lepidoptera, Pyralidae) by various predators (Fig. 6.23). Adults of the coccinellid *Coleomegilla maculata* consumed the entire egg mass, leaving only the basal and lateral parts of the egg chorion. The staphylinid beetle *Stenus flavicornis* chewed around the edges of an egg mass and killed many more eggs than were actually consumed. Larvae of the lacewing *Chrysopa* sp. left distinctive feeding holes in the egg chorion. The heteropteran bug *Orius insidiosus* did not completely consume the egg, and extensive melanisation occurred near the site of attack. The phytoseiid mite *Amblyseius* sp. also caused melanisation, but of more restricted extent. Andow (1992) used this information to interpret damage to sentinel egg masses in maize crops, and showed that *C. maculata* predation was greatest in plots established after ploughing, compared with *Chrysopa* predation, which was greatest in no-till plots. Hossain et al. (2002) used sentinel moth eggs of *Helicoverpa* spp. (live eggs attached to sections of stiff paper which were stapled to lucerne plants) to provide an index of egg predation intensity in plots of harvested and unharvested lucerne. To avoid egg hatch in the field, eggs less than 24 h old were used, and were transported to the field at 9 °C in

a portable refigerator. Egg cards were then retrieved from the field after 24 h' exposure to predation (Hossain et al., 2002). When prey are extremely small, scanning electron microscopy (SEM) can be used successfully to categorise characteristic predator-specific feeding traces in the laboratory, and then screen prey taken from the field to determine the incidence of predation by different species of predator. Using SEM in this way, Kishimoto and Takagi (2001) showed that staphylinid beetles of the genus *Oligota* were the main predators of spider mite eggs on pear trees in Japan. Feeding traces have also been used to identify the carabid, staphylinid and mammalian predators of winter moth (*Operophtera brumata*) pupae in oak woodland (East, 1974). Caterpillars of fruittree leafroller, *Archips argyrospila*, have a characteristic blackened and shrunken appearance after being fed on by carabid beetles, and this was used to estimate predation rates after examination of infested foliage (Braun et al., 1990). Similarly, spongy moth (*Lymantria dispar*) pupae with characteristic large, jagged wounds, were known to have been attacked by the carabid beetle *Calosoma sycophanta* (Fuester & Taylor, 1996). Examination of damaged egg masses of *Lymantria dispar* on cork oak showed cork dust and faecal pellets characteristic of oöphagous beetle larvae, *Tenebroides maroccanus* (Trogossitidae) (Villemant & Ramzi, 1995). Morrison et al. (2016) conducted laboratory feeding trials using field-collected predators and developed a classification system for different damage syndromes (complete chewing, incomplete chewing, stylet sucking, and punctured sucking). They then used this classification system to identify probable predators of field-deployed sentinel egg masses of the brown marmorated stink bug (*Halyomorpha halys*). Mummies of *Aphis gossypii* containing large ragged-edged holes were considered to have been attacked by coccinellid beetles (Colfer & Rosenheim, 2001). Predation marks on collected mines were used to identify which predators had attacked citrus leafminer *Phyllocnistis citrella* (Amalin et al., 2002). Ants, for example, slit open the mine and pull out the prey but lacewing larvae pierce the mine and suck out the fluid contents of the prey. For a few species, visual inspection reveals a clear difference between parasitised and unparasitised hosts that can be assigned to a particular species of parasitoid. For example, healthy pupae of the small white buttefly, *Pieris rapae*, are green, but those attacked by *Pteromalus puparum* are brown (Ashby & Pottinger, 1974). Pupae of the glasshouse whitefly, *Trialeurodes vaporariorum*, when attacked by the parasitoid *Encarsiaformosa*, turn from white to black after several weeks when the parasitoid pupates (Sunderland et al., 1992).

With the workers of some predatory ant species it is possible, using forceps, to gently remove food items (Ibarra-Núñez et al., 2001) from the jaws of the ants as they return to the nest (Stradling, 1987). Rosengren et al. (1979), in a detailed study of wood ants (*Formica rufa* group) in Finland, used this method to investigate the diet of *Formica polyctena* (Table 6.4). Vogt et al. (2001) exposed the underground foraging trails of fire ants (*Solenopsis invicta*) to collect the ants together with the material they were carrying. Unfortunately, the manual method of food item collection disturbs the ants, and some prey items may be missed. Skinner (1980) overcame these problems when studying the feeding habits of the wood ant *Formica rufa* (Formicidae), by using a semi-automatic sampling device which collected the ant 'booty' as it was carried back to the nest. With this method, an ant nest is surrounded by a barrier which forces the ants to use the exit and entry ramps provided. The incoming ants are then treated in either of two ways:

1. They are periodically directed through a wooden box with an exit-hole sufficiently large to allow the ant, but not its booty, to pass through. Prey left in the box may then be retrieved and identified.
2. They are allowed to fall off the end of the entry ramp into a solution of 70% alcohol.

As the latter treatment kills the ants it can be used for short periods only.

The remains of the prey of larval ant lions (Neuroptera: Myrmeleontidae) can be located, either buried in the predator's pit, or on the sand

Table 6.4 Pooled data from four nests showing number of prey items (*n*) expressed as a percentage of total items (%) carried to the nest of *Formica polyctena* during two periods in 1978

	30 June–3 July		27 July–31 July	
	n	%	*n*	%
Homoptera (mostly aphids)	135	20.4	15	2.4
Adult Diptera (mostly Nematocera)	122	18.4	147	23.4
Sawfly larvae (Tenthredinidae)	73	11.0	50	7.9
Lepidoptera larvae	74	11.2	43	6.8
Unidentified carrion	53	8.0	78	12.4
Adult Coleoptera (mostly Cantharidae)	51	7.7	34	5.4
Ant workers	33	5.0	52	8.3
Ant reproductives	2	0.3	38	6.0
Lumbricidae (number of pieces)	23	3.5	21	3.3
Homoptera Auchenorrhyncha	19	2.9	22	3.5
All other groups	76	11.5	129	20.5
Total number of items	661	99.9	629	99.9

Source Rosengren et al. (1979). Reproduced by permission of the International Organisation for Biological and Integrated Control

surface close to the pit, and can be identified, as shown by Matsura (1986). The diet of first-instar larvae of *Myrmeleon bore* was far less varied than that of second- and third-instar larvae.

Food items may be either observed in situ on, or manually collected, from the webs of spiders. This method was used by Middleton (1984) in a study of the feeding ecology of web-spinning spiders in the canopy of Scots Pine (*Pinus sylvestris*), and by Sunderland et al. (1986b) when measuring predation rates of aphids in cereals by money spiders (Linyphiidae). Alderweireldt (1994) collected linyphiid spiders that were carrying prey in their chelicerae, and prey remains were collected from their webs, in Belgian maize and ryegrass fields. Jmhasly and Nentwig (1995) made regular daytime surveys of spider webs in a field of winter wheat. Each web was visited at 45-min intervals in an attempt to record prey cadavers in the webs before spiders had time to discard the remains. The timing of web observations can be optimised in relation to environmental conditions and spider biology. Miyashita (1999), for example, visited webs of *Cyclosa* spp. in hedgerows between 1100 and 1300, because about half of the webs were destroyed by 1600 (due to the action of wind and damage by prey) and webs were rebuilt only at night. Pekár (2000) collected entire webs of the theridiid spider *Theridion impressum*, and captured prey were picked out and identified later in the laboratory. In all these studies, examination of webs was confined to the daylight hours, because small prey items in delicate webs on or near the ground cannot be recorded reliably at night. The prey spectrum at night could be different from that which has been recorded during the daytime, and so techniques need to be developed to investigate nocturnal predation.

Some ant species make refuse piles which could be examined to determine their diet. Caution is needed here, however, since Vogt et al. (2001) found that the refuse piles of *Solenopsis invicta*, which were dominated by remains of Coleoptera, were entirely different from material seen to be carried into the nest, which was dominated by larvae of Lepidoptera. Prey and hosts of fossorial wasps, such as Sphecidae and Pompilidae, may be analysed by examining the prey remains within the nest. Larval provisioning by the wasp *Ectemnius cavifrons*, a predator of hover flies, was investigated by Pickard (1975), who removed prey remains from the terminal cell of a number of burrows in a nest. Other fossorial wasps may be investigated using artificial nests: i.e., already hollow plant stems, or pieces of

dowelling or bamboo that have been hollowed out by drilling (Cooper, 1945; Fye, 1965; Bohart, 1966; Krombein, 1967; Parker & Danks, 1971; Southwood & Henderson, 2000). The stem or dowelling is split, and then bound together again, with, for example, string or elastic bands. The binding may subsequently be removed to allow examination of the nest contents. Details of one of the aforementioned methods, together with photographs of artificial nests and individual cells, are contained in Krombein (1967). Thiede (1981) describes a trap-nesting method involving the use of transparent acrylic tubes. An entrance trap that separates yellowjacket wasps (*Vespula* spp.) entering and leaving the nest has been devised. Those entering can be diverted into a gassing chamber and anaesthetised with carbon dioxide to enable their prey load to be removed and identified. The wasps treated in this way eventually recover, and 30–60 wasps can be sampled per hour (Harris, 1989). The large tube of an entrance trap can be fitted to the nest entrance at night, using black polythene and soil. Then, an inner collecting container (with integral funnel) can be inserted into the large tube during daytime to trap returning foragers (Harris & Oliver, 1993).

6.3.4 Tests of Prey and Host Acceptability

Information on host or prey range in a natural enemy species can be obtained by presenting the predators or parasitoids, in the laboratory, with potential prey or hosts, and observing whether the latter are accepted (e.g., Goldson & McNeill, 1992; Schaupp & Kulman, 1992, for studies on parasitoids). For visually hunting predators, it may be possible to use video-imagery techniques that enable the prey stimulus to be controlled with precision, and standardised. Clark and Uetz (1990) showed that the jumping spider *Maevia inclemens* did not discriminate between live prey and a simultaneous video image of prey displayed on Sony Watchman micro-television units. These spiders stalked and attacked the televised prey. Arachnids have flicker fusion frequency values in a similar range to that of humans, and so images are percieved as moving objects, rather than as a series of static frames. Some insects, however, have critical flicker fusion frequency values greatly in excess of 60 Hz (the human range is 16–55 Hz and arachnids span the range 10–37 Hz) and would not be good subjects for this methodology.

Simple laboratory assessments of prey and host acceptability should always be treated with caution, because of the artificial conditions under which they are conducted (Greenstone, 1999). They can only indicate *potential* trophic relationships, and other factors need to be taken into consideration when extrapolating to the field situation. For example, it is necessary to establish that the natural enemy being investigated actually comes into contact with the potential prey or host species under natural conditions. The predator or parasitoid may only be active within the habitat for a limited period during the year, and this may not coincide with the presence of vulnerable stages of the prey, or host, in that habitat. In order to avoid testing inappropriate predator/prey or parasitoid/host combinations it is thus advisable to establish whether there is both spatial and temporal synchrony before carrying out laboratory acceptability tests (Arnoldi et al., 1991). The placing of insects in arenas, such as Petri dishes and small cages, also raises serious questions regarding the value of the method. Confining potential prey or hosts in such arenas alters prey and host dispersion patterns, and, in particular, it is likely to reduce the opportunities for prey to escape from the natural enemy, so increasing the risk of false positives being recorded. Some insect species may rarely, if ever, be attacked in the field by a particular natural enemy species, because they are too active to allow capture by the latter. For some types of predator, such as web-building spiders, prey acceptability tests can be carried out in the field. The same strictures about testing appropriate prey types still apply to field testing, but at least the behaviour of the predator is natural and unconstrained. Henaut et al. (2001), for example, used an aspirator to gently blow live potential prey organisms into the orb webs of spiders in a coffee plantation.

They used prey types that were abundant in the plantation and recorded the extent to which the web retained the prey, as well as capture and consumption rates by the spiders. Some of the problems of testing prey preferences in the laboratory can be overcome by providing more architecturally complex arenas e.g., by adding stepped levels or obstacles (Carter & Dixon, 1982) or even using whole plants (e.g., Carter et al., 1984).

Laboratory studies are particularly useful in determining prey size choice, helping to determine an upper limit to the size of prey that the predator is capable of tackling (Greene, 1975; Loreau, 1984b; Digweed, 1993; McKemey et al., 2001), and, sometimes, a lower limit below which single, unaggregated prey are ignored (Greene, 1975). Finch (1996) showed that carabids (Fig. 6.24) with mandibles above a certain size were incapable of manipulating, and therefore of consuming, the eggs of the cabbage root fly, *Delia radicum*. These limits on prey size can be particularly relevant in cases where size determines the species range or life stage that will be attacked by a polyphagous predator. McKemey et al. (2001) found that the carabid *Pterostichus melanarius* had a preference for smaller slugs within laboratory arenas, but this result was not supported by choice experiments within a crop. Within the structurally complex habitat of a crop of wheat no slug size preferences were found (McKemey et al., 2003), apparently because the smallest slugs had greater access to refugia from the beetles within cut wheat stems and clods of soil. Again, therefore, laboratory studies can provide misleading data; they can show which sizes of prey the predators are capable of killing, but not necessarily the preferences the predators have in the field.

Predators are often also scavengers. A predator that is known to consume a particular prey type may be (with respect to that prey type) a

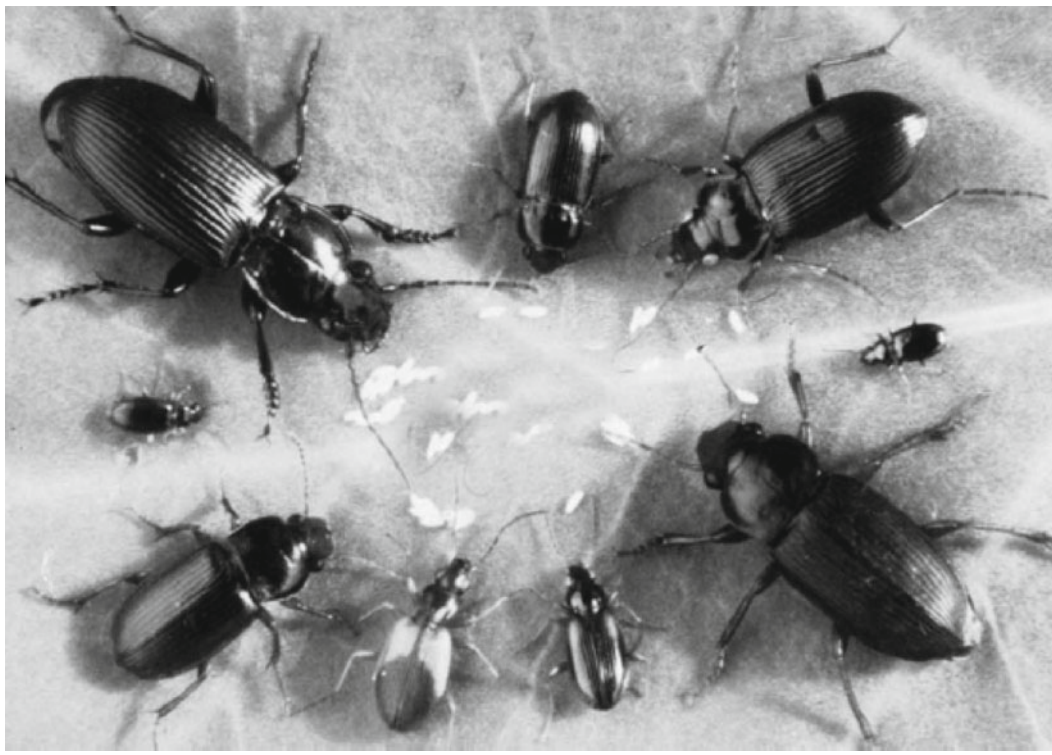

Fig. 6.24 Size range of carabid beetles in relation to predation of the eggs of cabbage root fly, *Delia radicum. Source* Finch (1996). Reproduced by permission of The Royal Entomological Society

scavenger, rather than a true predator. This is an important distinction if the impact of predation on the prey species is being inferred from the post-mortem examination of field-collected predators. Laboratory studies are appropriate for determining whether a predator has the physical and behavioural capacity to kill a particular potential prey species (Greenstone, 1999; Sunderland, 2002). Sunderland and Sutton (1980) showed that eight species of predator that regularly consumed woodlice (Isopoda) in the field (as determined from serological investigations), were, in fact, unable to kill even tiny woodlice in laboratory tests, and were therefore unlikely to have been predators of woodlice in the field. Halaj and Cady (2000) frequently observed harvestmen (Opiliones) eating earthworms (Lumbricidae) in the field, but laboratory tests showed that even the largest species of harvestman was incapable of killing earthworms of a size that were regularly consumed in the field. Therefore, harvestmen in the field were probably scavenging. Some species of ant that were shown (by ELISA) to contain remains of the olive moth, *Prays oleae*, were thought to have obtained these remains primarily by scavenging (Morris et al., 2002).

Even when a parasitoid stabs the host with its ovipositor, the host may still be rejected for oviposition (McNeill et al., 2000; Sect. 1.9). It is therefore important that egg release is confirmed during host acceptability tests involving parasitoids. In a few cases this can be done by observing the behaviour of the parasitoid; for example, in egg parasitoids that conspicuously rub their ovipositor over the surface of the host when they deposit a marking pheromone (Rabb & Bradley, 1970). Otherwise, it can be done either by dissecting hosts shortly after exposure to parasitoids, to locate eggs within the host's body (Chap. 2), or by rearing hosts until parasitism becomes detectable. Furthermore, just because an insect species is accepted for oviposition, does not mean that the species is suitable for successful parasitoid development. Under laboratory conditions many parasitoids will, if given no alternative, oviposit in or on unsuitable 'hosts', but subsequent monitoring of the progeny of endoparasitoids will show that they have been killed by the host's physiological defences (Sect. 2.10.2), while for ectoparasitoids, eggs may hatch but fail to develop successfully due to the host being biochemically unsuitable (I. C. W. Hardy, personal communication).

On the other hand, oviposition in unsuitable hosts is not always a laboratory artefact; it does occur in some host–parasitoid associations (Heimpel et al., 2003; Condon et al., 2014; Abram et al., 2016, 2019), including in new interactions between native and exotic species (Heimpel et al., 2003; Abram et al., 2014; Gariepy et al., 2019). In these situations, it is critical to directly observe the parasitoid's behaviour (i.e., whether it oviposits in the host or not) to avoid misinterpretations that can arise from 'black-box' tests. If parasitoid behaviour is not observed whilst in contact with the host, resultant lack of parasitoid offspring emergence could be due to either lack of acceptance by the maternal parasitoid, or poor host suitability for offspring development once accepted for oviposition. For example, Abram et al. (2014) showed, first in the laboratory using filming and rearing, that the native North American parasitoid *Telenomus podisi* readily oviposited in eggs of the invasive stink bug *Halyomorpha halys* but parasitoid offspring did not successfully develop (other studies have since shown that very low levels of successful development of native parasitoids does occur in some locations; e.g., Dieckhoff et al., 2017). Similar results were found with egg parasitoids native to Europe (Haye et al., 2015). Two follow-up studies used molecular tools and found that 20–55% of field-collected *H. halys* egg massed in invaded regions contained the DNA of native parasitoids (Konopka et al., 2018; Gariepy et al., 2019), but parasitoid emergence was negligible, indicating that unsuccessful parasitism of *H. halys* eggs by native egg parasitoids is common in nature. Thus, laboratory results accurately predicted parasitoid host acceptance behaviour in the field, and conducting direct observations of parasitoid behaviour in laboratory suitability tests was necessary to make this prediction.

Because such unsuccessful parasitism may contribute to egg limitation of the maternal

female parasitoid, may lead to the death of the parasitoid offspring, and may also kill the host in some cases, its ecological relevance and contribution to biological control cannot be discounted (Condon et al., 2014; Abram et al., 2016, 2019; Kaser et al., 2018). In host–parasitoid associations where this is a possibility, laboratory trials should always include both direct observations of parasitoid behaviour and unattacked control hosts run in parallel with hosts exposed to parasitoids to test the possibility that hosts are being killed by unsuccessful parasitoid attack (with the possibility of mortality from host feeding also in mind, depending on the species) (Abram et al., 2016). Abram et al. (2019) review the ways that parasitoids can reduce the fitness of their hosts without producing offspring themselves (i.e., 'nonreproductive effects'), provide methods of measurement, and discuss their relevance to biological control.

When the introduction of an exotic natural enemy species into a new geographical area is being contemplated (in a classical biological control programme), it is important to determine whether the natural enemy is potentially capable of parasitising or preying on members of the indigenous insect fauna (Sect. 7.4.4). This can only be done safely through laboratory acceptability tests. The braconid parasitoid *Microctonus hyperodae* was collected from South America and screened for introduction into New Zealand as a biological control agent of the weevil *Listronotus bonariensis*, a pest of pasture (Goldson & McNeill, 1992). Whilst in quarantine, the parasitoid was exposed to as many indigenous weevil species as possible, giving priority to those of a similar size to the target host. In the tests, 25–30 weevils of each species were exposed to a single parasitoid for 48 h in small cages, after which the weevils were held in a larger cage to await the emergence of parasitoids. In a second series of trials, the parasitoids were given a choice of target hosts (*L. bonariensis*) and test weevils in the same cages. It is important to carry out such a choice test, since in no-choice laboratory trials natural enemies may attack insect species which they would normally ignore in field situations, thereby giving a false impression of the natural enemy's potential behaviour following field release. However, choice tests are not always relevant, depending on whether the species being tested would be expected to encounter both host species in the same area in nature. Suggested methodology for such non-target host range testing is discussed at length elsewhere (Bigler et al., 2006; van Lenteren et al., 2006; Sect. 7.4.4).

6.3.5 Examination of Hosts for Parasitoid Immatures

The principle behind this technique is that by examining (or dissecting, in the case of endoparasitoids) field-collected hosts, parasitoid immatures, or their remains, located upon or within hosts, they can be identified to species, thus providing information on the host range of parasitoids, and the species composition of parasitoid complexes. Unfortunately, the requirement that the parasitoid taxa involved be identified can rarely be satisfied. Few genera, and even fewer higher taxa, have a published taxonomy (i.e., keys and descriptions to the species developed for their immature stages, Sect. 6.2.9), and, where such taxonomies are available, they are rarely complete. Nevertheless, informal taxonomies can be developed in conjunction with the rearing method described in the next section (Sect. 6.3.6), by associating immature stages, or their remains, with reared adult parasitoids. Progress has, however, been made using PCR to identify parasitoid remains within hosts for up to three weeks after parasitoids have emerged (Gariepy et al., 2014; Varennes et al., 2014).

6.3.6 Rearing Parasitoids from Hosts

One of the most obvious ways of establishing host–parasitoid trophic associations is to rear the parasitoids from field-collected hosts. Smith (1974), Gauld and Bolton (1988), Shaw (1997) give general advice, and Jervis (1978), Starý (1970) describe methods of rearing parasitoids of leafhoppers and aphids, respectively. The ease

with which this can be done varies with parasitoid life-history strategy. It is usually far easier to rear idiobionts from eggs, pupae or paralysed larvae, than it is to rear koinobionts (Sect. 2.9), since the hosts of koinobionts usually require feeding. When supplying plant material to phytophagous hosts of koinobionts during rearing, it is very important to ensure that the material does not contain individuals of other insect species from which parasitoids could also emerge, as this can lead to erroneous host–parasitoid records. The risk of associating parasitoids with the wrong hosts is particularly acute when parasitoids are reared from hosts in fruits, seed heads and galls, as these may contain more than one herbivore, inquiline or the host species. Such material should be dissected after the emergence of any parasitoids, so that the host remains can be located and identified, and the parasitoid–host association determined correctly.

Whenever possible, hosts should be reared individually in containers, so that emerging adult parasitoids can be associated with particular host individuals. This allows the recording of accurate data on any parasitoid preferences for particular host developmental stages or sexes, and it also avoids potential problems arising from the failure of the entomologist to distinguish between closely related host species. The choice of suitable containers will depend upon the host involved, but very often simple boxes, tubes or plastic bags will suffice (Starý, 1970; Smith, 1974; Jervis, 1978; Grissell & Schauff, 1990; Godfray et al., 1995; Shaw, 1997). The addition of materials such as vermiculite, plaster of Paris, or wads of absorbent paper, to rearing containers is recommended to avoid problems such as growth of moulds associated with the accumulation of excessive moisture.

Some parasitoids, including several Braconidae (Shaw & Huddleston, 1991), cause changes in host behaviour that often result in the movement of parasitised individuals from their normal feeding sites prior to death, as was recorded, for example, for the aphid parasitoid, *Toxares deltiger* (Powell, 1980). A significant proportion of the mummies formed by several aphid parasitoid species occur some distance away from the aphid colony, and even away from the food plant. They tend to be missed during collection, leading to inaccuracies in measurements of parasitism, and underestimation of the size of the parasitoid complex (Powell, 1980). As an insurance against the possible complicating effects of parasitoid-induced changes in host behaviour, it is advisable to collect and rear a random sample of apparently healthy hosts, in addition to obviously parasitised individuals. With aphids, it is also advisable to rear parasitoids from both live aphids and mummies, because some hyperparasitoids oviposit into the mummy stage, whereas others oviposit into the larval parasitoid prior to host mummification (Dean et al., 1981).

Parasitoids may also be collected from the field after they have killed, and emerged from, the host. The larvae of some species leave the body of their host and pupate, either upon the host remains, or on surrounding vegetation. The host remains (for subsequent identification) and the parasitoid pupae (which may be in cocoons or the exuvium of the last larval instar) may be collected, and parasitoids reared from the latter in individual containers. Where gregarious species are concerned, the pupae from a particular host individual should be kept together.

The importance of keeping detailed records of all parasitoid rearings cannot be overemphasised. At the time of collection from the field, the identity and developmental stage of the host should be recorded, along with habitat and food plant data, location and date (Gauld & Bolton, 1988). Where possible, the date of host death, together with that of parasitoid emergence, should be recorded. When adult parasitoids are retained as mounted specimens, the remains of the host and parasitoid pupal cases and cocoons should also be retained, to aid identification. Such remains are best kept in a gelatin capsule, which can be impaled on the pin of the mounted parasitoid. If a previously unrecorded host–parasitoid association is recorded during rearing, it is very important to provide 'voucher' specimens for deposition in a museum collection.

In order to ensure, as far as possible, that all the species in the parasitoid complex of a host are

reared, samples of parasitised hosts need to be taken from a wide area, from both high- and low-density host populations, from a variety of host habitats, and over the full timespan of the host life cycle, or at least the timespan of the host stage that one is interested in (for example, the pupal stage, if only pupal parasitoids are the subject of interest). A parasitoid species may be present in some local host populations, but not in others. Its absence may be due to climatic unsuitability, or because of an inability of the parasitoid to locate some populations. Ecologists refer to the constancy of a parasitoid species (Zwölfer, 1971): the probability with which a species may be expected to occur in a host sample. Constancy is expressed as the percentage, or the proportion, of samples taken that include the species, e.g., see Völkl and Starý (1988). Host species with a wide geographical distribution require more extensive sampling to reveal the total diversity of their parasitoid complex than do host species with a restricted distribution (Hawkins, 1994). One can reasonably assume that the relationship between the extent of the host's distribution and parasitoid species richness is linear (Tylianakis et al., 2006).

Major temporal changes may occur in the species composition of a parasitoid complex. Askew and Shaw (1986) refer to the example of the lepidopteran *Xestia xanthographa*. Samples of larvae of this host taken 10 weeks apart yielded very different species of parasitoid (Table 6.5). Another factor to consider is sample size. The probability of a parasitoid species being reared from a host will depend on the percentage parasitism inflicted, and also on the size of the host sample taken. The larger the sample of hosts from a locality, the greater the probability that all the parasitoid species in the local complex will be reared. This relationship is probably asymptotic, and sample sizes of 1000 hosts, or larger, provide good estimates of parasitoid species richness that are not so strongly dependent on sample size (B. A. Hawkins, personal communication). Martinez et al. (1999) discuss the amount of sampling effort required to reveal the structural properties of parasitoid food webs.

As well as providing information on host range, parasitoid complex, and community structure, rearing of parasitoids can also yield valuable data on parasitoid phenologies. For example, by noting the times of emergence of

Table 6.5 Parasitism of *Xestia xanthographa* collected near Reading, UK, on two dates during spring 1979. The figures take no account of larval-pupal parasitoids.

Numbers of unparasitised larvae		Sampling dates	
		2 March	12 May
Numbers of larvae parasitised by		188	50
Tachinidae	*Periscepsia spathulata* (Fallén)	24	0
	Pales pavida (Meigen)	1	0
Braconidae	*Glyptapanteles fulvipes* (Haliday)	8	0
	Cotesia hyphantriae Riley	1	0
	Meteorus gyrator (Thunberg)	1	0
	Aleiodes sp. A	46	0
	Aleiodes sp. B	10	0
Ichneumonidae	*Hyposoter* sp	1	0
	Ophion scutellaris Thomson	0	6
Total larvae sampled		280	56
Percentage parasitism		33	11

Source Askew and Shaw (1986). Reproduced by permission of Academic Press Ltd.

larval parasitoids from the hosts, and the time of adult emergence, Waloff (1975), Jervis (1980b) obtained valuable information on the timing and duration of adult flight period, and on the incidence of diapause, in several species of Dryinidae and Pipunculidae.

Rearing can also be a valuable source of material for taxonomic study. If a larval taxonomy of the parasitoids is to be developed (Sect. 6.2.9), association of parasitoid adults with larvae can usually only be achieved reliably through rearing. By associating reared adults with their puparial remains, Benton (1975), Jervis (1992), developed a larval taxonomy for a number of Pipunculidae. Some parasitoids are more easily identified through examination of their larval/puparial remains than they are through examination of the adult insects (Jervis, 1992). The molecular analysis of hosts to detect immature parasitoids is discussed in Sect. 6.3.11.

6.3.7 Faecal Analysis

For predators that have faeces which contain identifiable prey remains, faecal analysis can be used to estimate dietary range. This method has been applied to aquatic insects (Lawton, 1970; Cloarec, 1977; Thompson, 1978; Folsom & Collins, 1984). Folsom and Collins (1984) collected larvae of the dragonfly *Anax junius* and kept them until their faecal pellets were egested. The pellets were subsequently placed in a drop of glycerin-water solution on a microscope slide and teased apart. Prey remains were identified by reference to faecal pellets obtained through feeding *Anax* larvae on single species of prey, by examination of whole prey items and by examination of previously published illustrations of prey parts. The data obtained were used to compare the proportion of certain prey types in the diet, with the proportions found in the aquatic environment. Carabid beetles often consume solid fragments of their prey (Sect. 6.3.8), and may retain these in the gut for days or weeks before defaecation of the meal is completed (Young & Hamm, 1986; Sunderland et al., 1987a; Pollet & Desender, 1990). This makes field-collected carabids suitable candidates for faecal analysis in the laboratory. Unless there is some reason for the beetles to be retained alive (e.g., they may be of an endangered species), gut dissection (Sect. 6.3.8) is more convenient than faecal analysis as a means of assessing carabid diet. Other types of predator, however, may be very difficult to dissect, in which case faecal analysis can be very useful. Phillipson (1960) examined 1367 faecal pellets from the harvestman *Mitopus morio* (Opiliones), and was able to determine the relative contribution of plant and animal material to its diet. Burgess and Hinks (1987) checked the faeces of several hundred field-collected crickets (*Gryllus pennsylvanicus*) and found that 0–28% (depending on collection site and season) had been feeding on adult flea beetles (*Phyllotreta cruciferae*), judging by characteristic remains of elytra, antennae and metathoracic legs. These authors had earlier determined, from laboratory experiments (Sect. 6.3.4), that *G. pennsylvanicus* is a voracious predator of *P. cruciferae*. Rate of production of faeces has also been used in an attempt to estimate predation rates by ladybirds (Honěk, 1986), and to estimate the time of the most recent meal in the field by carabids (Young & Hamm, 1986).

6.3.8 Gut Dissection

If a predator is of the type that ingests the hard, indigestible, parts of its prey, simple dissection of the gut might easily disclose the prey's identity, as is indeed the case for a number of predators. The main advantages of this method for investigating trophic relationships are the simplicity of equipment required and the immediacy of results.

Dissection is usually performed using entomological micropins or fine watchmaker's forceps, which are used to remove and transfer the digestive tract (the crop, proventriculus, mid gut and hind gut, Chap. 2) to a microscope slide where it can be teased apart and the prey fragments identified. As with faecal analysis (Sect. 6.3.7), the prey remains found in field-collected predators can be compared with those

on reference slides prepared by feeding a predator with a single, known type of prey. Predators need to be killed as soon as possible after collection, otherwise the gut contents may be lost due to the predator defecating or regurgitating. *Sialis fuliginosa* loses a proportion of its gut contents by regurgitation when placed in preservative (Hildrew & Townsend, 1982) and many carabids will regurgitate if handled roughly. Not all invertebrates lose gut contents in this manner, but this possibility must be borne in mind when carrying out gut dissection. Some aquatic predators collected in nets continue feeding in the net, so need to be narcotised immediately upon capture, e.g., *Chaoborus* larvae (Pastorok, 1981). Similar precautions are needed for samples taken from terrestrial pitfall traps.

Gut dissection has been used to study the diet of a range of predators: (a) aquatic insects (Bay, 1974; Fedorenko, 1975; Hildrew & Townsend, 1976, 1982; Pastorok, 1981; Woodward & Hildrew, 2001, 2002), (b) carabid beetles (Hengeveld, 1980; Sunderland & Vickerman, 1980; Dennison & Hodkinson, 1983; Chiverton, 1984; Pollet & Desender, 1986; Luff, 1987; Sunderland et al., 1987a; Dixon & McKinlay, 1992; Holopäinen & Helenius, 1992; Holland et al., 1996), (c) staphylinid beetles (Kennedy et al., 1986; Chiverton, 1987), (d) coccinellid beetles (Eastop & Pope, 1969; Ricci, 1986; Triltsch, 1997), (e) earwigs (Dermaptera) (Crumb et al., 1941; Skuhravý, 1960), (f) harvestmen (Opiliones) (Dixon & McKinlay, 1989), and (g) centipedes (Chilopoda) (Poser, 1988). When dissecting carabid beetles, it can be worthwhile to examine not only the crop, but also the proventriculus and rectum, because characteristic prey fragments (such as the S-shaped chaetae of lumbricid earthworms) can lodge here for longer periods than in the crop (Pollet & Desender, 1990). Sunderland (1975) examined the crop, proventriculus and rectum of a variety of predatory beetles (Carabidae, Staphylinidae). In a similar study, the crop and the hindgut of two New Zealand carabid species were placed separately on microscope slides and examined in 50% glycerol at up to 400 × magnification. Food remains were identified, as far as possible, using microscopical preparations from reference collections (grass and weed seeds, other plant material, invertebrates from a range of sampling methods) for the areas where the carabids were caught (Sunderland et al., 1995b). These methods were also used to investigate diet of the carabid *Pterostichus versicolor* from heathland. Beetles were caught in pitfall traps and dissected. Slides were prepared of the fore- and the hindgut. Reference slides were made by feeding beetles with known prey, and also by macerating known arthropods (Bruinink, 1990). Gut contents of freshwater aquatic predatory invertebrates can also be identified from reference slides (Woodward & Hildrew, 2002).

Although, in studies such as the above, many fragments found in the gut could be placed in general prey categories, identification of prey remains to species has often only been possible if distinctive pieces of prey cuticle (e.g., aphid siphunculi) remained intact. Recognisable fragments generally found in the guts of carabid and staphylinid beetles include: chaetae and skin of earthworms; cephalothoraces of spiders; claws, heads and/or antennae from Collembola; aphid siphunculi and claws; sclerotised cuticle, mandibles and legs from beetles and the head and tarsal claws of some Diptera (Fig. 6.25). Hengeveld (1980), Chiverton (1984), in their studies of Carabidae, also had to group most dietary components, assigning only a few prey remains to species. Pollet and Desender (1988) were able to identify, to species, the remains of some Collembola in the guts of carabid beetles. The efficiency of detecting large Collembola was 67% (based on recovery of Collembola mandibles), and a semi-quantitative analysis of consumption was therefore possible. Similarly, Dixon and McKinlay (1989) identified, to species, some of the aphid remains from the guts of harvestmen, and were able to estimate that some individual *Phalangium opilio* contained the remains of at least five aphids.

An obvious difficulty with the gut dissection method is the digestion of prey prior to dissection. The abilities of the different investigators to recognise the prey remains will influence

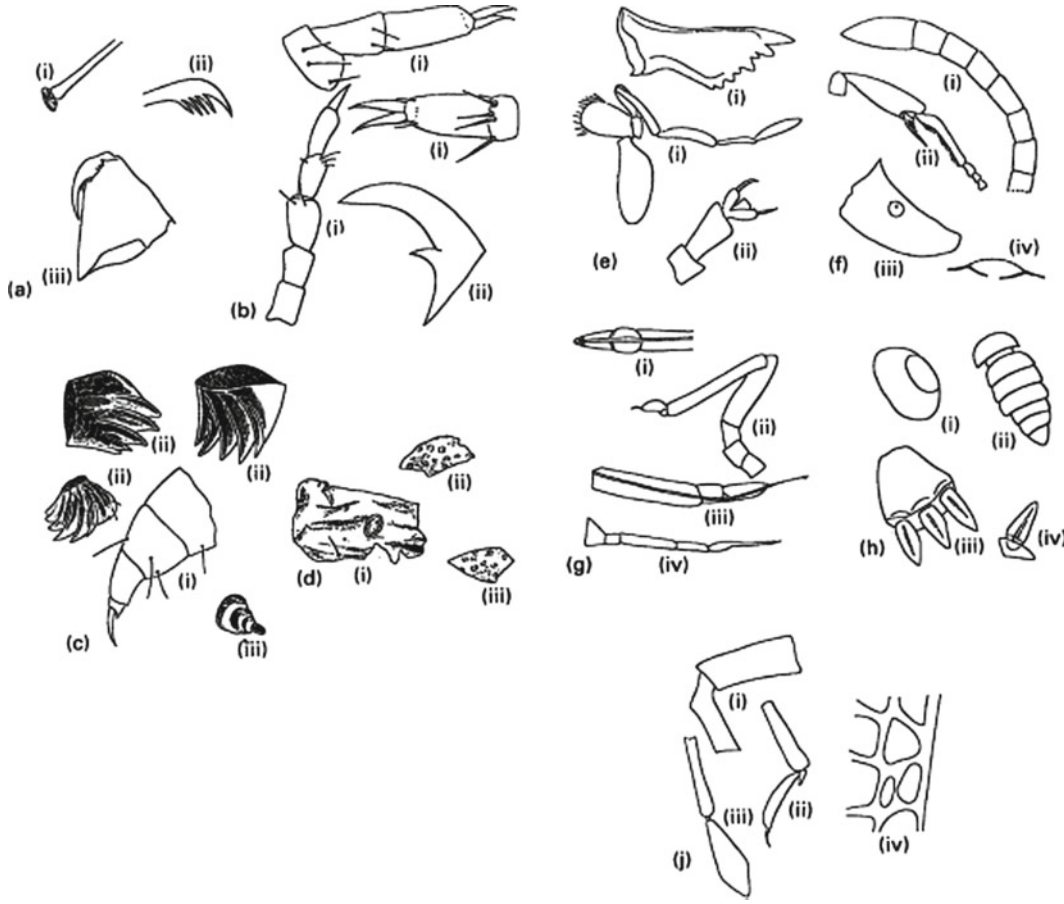

Fig. 6.25 Examples of prey fragments found in dissections of carabid beetle guts: **a** lycosid spider, (i) bristle, (ii) claw, (iii) chelicera; **b** carabid or staphylinid beetle larva, (i) legs, (ii) mandible; **c** lepidopterous (?) larva, (i) leg, (ii) mandibles, (iii) unidentified part; **d** fragment of exoskeleton of (i) lepidopterous (?) larva, (ii) heteropteran bug; e components of ant's mouthparts (i) and tarsus (ii); **f** ant's (i) antenna, (ii) leg, (iii) exoskeleton (fragment), (iv) ocellus, **g** components of aphid's (i, iii) mouthparts, (ii) leg, (iv) antenna; **h** bibionid fly, **i** antennal segment, (ii) antenna, (iii, iv) parts of leg; **j** *Acalypta parvula* (Hemiptera: Tingidae), (i, ii) parts of leg, (iii) part of antenna, (iv) fragment of forewing. *Source* Hengeveld (1980). Reproduced by permission of E. J. Brill (Publishers) Ltd

comparative studies, as will the ability to distinguish between fragments from the same or different individuals within a prey species or group.

Although the gut contents of predator species probably derive mainly from predation, carrion-feeding (scavenging) is also known to take place in some species. Therefore, a knowledge of carrion availability may also be useful in predicting the diet of a species. While scavenging is clearly problematic in a study of the effects of predation on pest population dynamics, it is not a problem for studies that set out to assess the role of alternative animal foods in sustaining predator populations (Symondson et al., 2000). Alternative foods may help to sustain generalist predators in a crop when pest numbers are low, allowing the predators to survive, ready to feed on the pest when it becomes available (Murdoch et al., 1985).

The acquisition by a predator of prey materials through secondary predation (i.e., feeding upon another predator species which itself contains prey items) can also produce misleading results. Although countable remains of certain

ingested structures (e.g., the mandibles of beetles or the shells of slugs) may provide some quantitative information, these parts of the prey may well be avoided by the predators. Whether they are eaten, even by a predator that is not a fluid-feeder and which consumes more solid remains, may depend upon the size of the indigestible parts (biasing the data towards small prey) or the state of hunger of the predator. An awareness of these problems is also required with the faecal analysis method described earlier.

The majority of predators are obligate fluid-feeders, and other predators are facultative fluid-feeders, with an unknown proportion of their ingestion confined to prey fluids. The following techniques (antibody, electrophoretic and DNA-based analyses) are suitable for investigating food consumption by fluid-feeders (Symondson, 2002b).

6.3.9 Antibody and Electrophoretic Techniques

Most predators are solely or partly fluid-feeders, and so their diet cannot be investigated using the aforementioned faecal analysis and gut dissection methods. Ecologists have, in the past (Loughton et al., 1963), used a variety of antibody and electrophoretic methods to study predation and parasitism. Since the publication of the first edition of this volume, however, these methods have been largely superseded by DNA-based techniques in recent years (Sect. 6.3.11). Thus, in this reduced section, we refer readers elsewhere for information on these techniques and their applications.

Standard methodologies for production and use of monoclonal antibody are described in Blann (1984), Goding (1986), Liddell and Cryer (1991), Liddell and Weeks (1995). Examples of the application of antibody techniques to determining natural enemy trophic relationships can be found in Symondson et al. (1996, 2000).

Variations on ELISA techniques are reported and described by Voller et al. (1979), and references dealing with their entomological applications are cited in Sunderland (1988), Greenstone (1996), Symondson and Hemingway (1997), Symondson (2002a). Examples of applications of ELISA in predation studies can be found in Fichter and Stephen (1979), Ragsdale et al. (1981), Crook and Sunderland (1984), Lövei et al., (1985), Service et al. (1986), Sunderland et al. (1987a), Greenstone and Morgan (1989), DuDevoir and Reeves (1990), Cameron and Reeves (1990), Hagler et al., (1992b), Hagler and Naranjo, (1994), Symondson et al. (1996), Symondson et al. (2000), Bohan et al. (2000). ELISA has also been applied to the investigation of parasitoids (Stuart & Burkholder, 1991; Allen et al., 1992; Stuart & Greenstone, 1996; Keen et al., 2001).

Many texts are available which deal with the general methods and techniques used in electrophoresis, e.g., Chrambach and Rodbard (1971), Gordon (1975), Sargent and George (1975), Hames and Rickwood (1981), Richardson et al. (1986), Pasteur et al. (1988), Hillis and Moritz (1990), Quicke (1993). The reader is referred to these texts for detailed descriptions and experimental protocols and to Menken and Ulenberg (1987), Loxdale and den Hollander (1989), Loxdale (1994), Symondson and Liddell (1996), Symondson and Hemingway (1997) for reviews of the application of electrophoretic methods in agricultural entomology.

6.3.10 Labelling of Prey and Hosts

Potential prey can be labelled with a chemical that remains detectable in the predator or parasitoid (Southwood & Henderson, 2000). By screening different predators within the prey's habitat for the presence of the label, the species composition of the predator complex can be determined.

Various labels are available for studying predation and parasitism. These include radioactive isotopes, stable isotopes, rare elements and dyes (usually fluorescent), which are introduced into the food chain, where their progress is monitored. The label is injected directly into the prey, or it is put into the prey's food source. Parasitoid eggs can be labelled by adding a marker to the

food of female parasitoids. Appropriate field sampling can then reveal whether suspected predators have eaten labelled prey, or whether suspected hosts have been oviposited in by a particular parasitoid species.

Collembola have been labelled with radioactive ^{32}P through their food. This did not affect Collembola survival rate, or the probability of predation by carabids. In a laboratory experiment, predation rates estimated by radiotracers were similar to those estimated from a comparison of Collembola survival in containers with and without predators (Ernsting & Joosse, 1974). *Helicoverpa armigera* and *H. punctigera* eggs were radiolabelled by injecting adult females with ^{32}P, and the fate of these eggs was then monitored in the food chain (Room, 1977). Both adults and larvae of the bean weevil, *Sitona lineatus*, were labelled with ^{32}P by allowing them to feed on broad bean plants which had their roots immersed in distilled water containing the radiolabel (Hamon et al., 1990). The labelled weevils were then exposed to predation by carabid beetles within muslin field cages, and the levels of radioactivity shown by the carabids subsequently measured using a scintillation counter. Greenstone (1999) lists six other studies in which radionuclides were used to detect consumption of pests by spiders. Breene et al. (1988) labelled mosquito larvae with radioactive ^{32}P and released them into simulated ponds where spiders were present. After 48 h spiders were removed to assess levels of radioactivity: 30–77% had eaten the labelled prey. Spiders were observed preying on the mosquito larvae by grasping them from beneath the surface of the water, pulling their bodies through the surface tension and then consuming them.

Labelling may also be used to investigate intraspecific trophic relationships, such as maternal care. Adult female lycosid spiders *(Pardosa hortensis*) were labelled for a month with radioactive tritium and leucine through their food (*Drosophila* flies). When they produced cocoons (which are carried attached to the spinnerets), the cocoons were removed and replaced with unlabelled cocoons, which the mothers adopted readily. Twenty days later, the spiderlings inside the cocoon were found to be labelled with radioactive tritium, and parts of the cocoon were labelled with radioactive leucine (an amino acid found in cocoon silk). This finding suggests that the mother periodically opened the cocoon, regurgitated fluid into it for the spiderlings, and then repaired the opening with fresh silk (Vannini et al., 1993). Monitoring of radioactive labels may be done using a Geiger counter or a scintillation counter, to measure α- and/or β-emissions (Hagstrum & Smittle, 1977, 1978), or by autoradiography (McCarty et al, 1980). In autoradiography, the sample containing the potentially labelled individuals is brought into contact with X-ray sensitive film, and dark spots on the developed film indicate the position of labelled individuals. However, although simpler to perform than either serological or electrophoretic methods, hazards to both the environment and the operator posed by radiolabelling mean that the method needs to be confined to laboratory studies or is used in the field such a way that the labelled insects can be safely and reliably recovered at the end of the experiment.

The cereal aphid *Sitobion avenae* was marked through its food with the stable isotope ^{15}N, samples being analysed with an elemental analyser coupled to a mass spectrometer (Nienstedt & Poehling, 2000) (Sect. 6.2.11). The label was detected successfully in a linyphiid spider (*Erigone atra*) and a carabid beetle, *Anchomenus dorsalis* (= *Agonum dorsale*), in laboratory tests. The isotope had no effect on the environment or on the behaviour of the labelled animals. Predators that had eaten two aphids had a significantly higher level of marker than those that had eaten one aphid, and the marker was detectable in predators for at least eleven days (Nienstedt & Poehling, 1995). ^{15}N can also be used to mark parasitoids (by rearing them on hosts that have been raised on a diet incorporating ^{15}N). Parasitoid adults are expected to retain the label throughout their life because nitrogen is a major constituent of fibrin and chitin (Steffan et al., 2001). Utilisation of stable isotopes of carbon and

nitrogen as natural tracers of nutrient flows in ecosystems is discussed in Sect. 6.3.1.

Rare elements such as rubidium or strontium may be used as markers, in a similar way to radioactive elements (Shepard & Waddill, 1976; Graham et al., 1978). For example, spongy moth larvae were successfully labelled with rubidium, and their phenology and survival were not affected. The label was retained for five days in these larvae, and adult carabids (*Carabus nemoralis*) eating larvae in laboratory trials acquired the tag. Rubidium concentration in beetles was positively correlated with number of larvae eaten, and negatively with the number of days since feeding (Johnson & Reeves, 1995). In such studies the path of the rare element through the food chain is monitored with the aid of an atomic absorption spectrophotometer. Unfortunately, while such markers may be retained for life, and self-marking is possible using labelled food sources, the equipment necessary for detection is expensive, both to purchase and to run, as well as requiring trained operators.

Prey can be labelled with rabbit immunoglobulin (IgG), then predators that feed on the labelled prey can be detected with a goat anti-rabbit IgG. The method is only appropriate for predators with chewing mouthparts (that ingest the exoskeleton of their prey), as only 30% of sucking predators were positive 1 h after feeding (Hagler & Durand, 1994).

Fluorescent dyes offer another means of marking prey. Hawkes (1972) marked lepidopteran eggs with such dyes during studies of predation by the European earwig, *Forficula auricularia*. An alcoholic suspension of dye was sprayed onto eggs, which were then eaten by the earwigs. Thereafter, the earwig guts were dissected out and examined under UV light. Although such dyes are useful, being both simple and inexpensive to use, they are relatively short-lived, and possible repellant effects, due to either the dye or the carrier, must be taken into account. The dye is usually voided from the predator gut within a few days. Hawkes (1972) found no evidence of dye retention in any of the internal structures of earwigs dissected. In a few specific cases, naturally coloured prey can be seen inside the gut of a living predator. For example, remains of citrus red mite (*Panonychus citri*) imparted a red colouration to the gut of the phytoseiid predator, *Euseius tularensis*, that did not fade until more than 8 h after ingestion (Jones & Morse, 1995). These authors devised a ten-point gut colouration rating scale to score the rate of decline of this natural label, after a meal, at a range of temperatures. Pigments from the mites, *Panonychus ulmi* and *Bryobia arborea*, can be seen in the guts of predators, such as lacewing larvae (Chrysopidae), that accumulate digestive wastes from large numbers of prey (Putman, 1965). Some of these ingested mite pigments fluoresce brilliantly in UV light. Pigments from very small numbers of mites can be detected by chromatography in small predators, such as anthocorid bugs, predatory thrips, and theridiid spiders, but, unfortunately, consumption of other prey can obscure the pigments (Putman, 1965). Fluorscent dye was also used to successfully track the movement and behaviour of the diamondback moth *Plutella xylostella* and its parasitoid *Diadegma semiclausum* in non-crop and crop areas (Schellhorn et al., 2008).

A potential alternative approach might be to live-stain host or prey organisms. Lessard et al. (1996) marked protists with a fluorescent DNA-specific stain (DAPI—2,4-diamadino-6-phenylindole; Sect. 3.4.1) which had no negative effects on their swimming behaviour or growth rate, and which was visualised easily in guts of their fish predators (larval walleye pollock, *Theragra chalcogramma*) using epifluorescence microscopy. It is not yet known whether this method could be used easily to study predation or parasitism (or, indeed, dispersal; Sect. 6.2.11) by arthropod natural enemies.

Major potential problems with the use of labels are secondary predation (Putman, 1965; Sunderland et al., 1997), scavenging and trophallaxis. The label may be recorded in several predatory and scavenger species within the prey's habitat, but only some of these may be directly responsible for prey mortality. Other food chains may also become contaminated (e.g., in the Johnson and Reeves (1995) study,

rubidium 'leaked' from both larvae and beetles in their frass), further adding to the confusion. An additional disadvantage is that, in most cases, prey must be handled, to some extent, to achieve the labelling, and this may disturb the system under investigation. Released prey may not have time to re-establish themselves, either in terms of spatial separation, or in their favoured microhabitats, possibly making them more vulnerable to predation and other biotic mortality factors (such as parasitism and disease).

6.3.11 DNA-Based Techniques

Molecular technology has rapidly permeated all areas of biology, and insect ecology is no exception. DNA techniques have almost entirely replaced electrophoresis, and other protein-based methodologies, in insect taxonomy for distinguishing between morphologically similar species, for constructing phylogenetic relationships, and for examining the stucture of populations and communities (Hrček & Godfray, 2014). This is mainly because DNA can provide, reliably, far more information, at all levels, than electrophoresis or any other previous approach (Symondson & Hemingway, 1997; Symondson, 2002b). DNA can be extracted from minute samples of insects (Hemingway et al., 1996), and the techniques are sufficiently fast for use in relation to pest management programmes (Loxdale et al., 1996). Useful manuals providing details of basic techniques can readily be found (e.g., Berger & Kimmel, 1987; Sambrook et al., 1989). Much is now known about how different regions of the genome evolve at different rates (Avise, 1994), and, hence, which target genes are most appropriate for examining differences at the level of the species, population or individual (e.g., Moritz et al., 1987; Liu & Beckenbach, 1992; Brower & DeSalle, 1994; Simon et al., 1994; Lunt et al., 1996; Caterino et al., 2000). A range of techniques has been developed, that are appropriate for use in entomological studies (Loxdale et al., 1996; Loxdale & Lushai, 1998). While the application of DNA techniques to the study of trophic interactions is still relatively new, it is growing exponenially. Here, we concentrate on techniques that have proved successful in studies of predation and parasitism, confining ourselves to studies which seek to detect and quantify interactions between organisms, rather than focusing on the genetics of the predators and prey or parasites and hosts. In general, methods for extracting DNA to detect parasitism and predation are the same as those in the many studies dealing with the molecular taxonomy of insects. A recent assessment of different methods for diet tracing, comparing molecular diagnostics with other techniques, can be found in Nielsen et al. (2018).

Detection of parasitoids within hosts

The rapid detection, identification and quantification of parasitoid early stages within their hosts is a major concern for practitioners of IPM who wish to avoid pesticide intervention wherever possible, and who also wish to monitor the spread of newly introduced biological control agents. Simple dissection is slow, labour-intensive and innacurate, and hence molecular techniques have been developed.

One of the simplest, fastest and least expensive molecular approaches is to use RAPD-PCR (Random Amplified Polymorphic DNA—Polymerase Chain Reaction) (Micheli et al., 1994; Rollinson & Stothard, 1994). The great advantage of this approach is that short random primers are selected initially, without the need for prior knowledge of target sequences. Although PCR conditions must be optimised, primers can be screened rapidly to find those that are capable of revealing reproducible, species-specific banding patterns on a gel for parasitoids within their hosts.

Black et al. (1992) detected parasitoids in aphids (*Diaeretiella rapae* in *Diuraphis noxia* and *Lysiphlebus testaceipes* in *Schizaphis graminum*), and the parasitoid patterns were species specific. Parasitoid DNA was detectable by six days after parasitism. PCR and arbitrary primers were used to distinguish between eight strains of *D. rapae*, three strains of *Aphidius matricariae*, and three other parasitoid species. Most primers give distinctly different patterns with different

species, and this should allow determination of the species involved in parasitism in field collections.

Concerns regarding reproducibility of the banding profiles have meant that RAPD-PCR is now rarely used for trophic analysis and many journals will no longer publish papers using this approach.

A more precise approach is to use primers for a target sequence within a specific gene. Much attention has been directed at genomic ribosomal gene clusters (rDNA), of which there are hundreds of tandemly repeated copies per cell. The level of conservation of these regions is such that universal primers are now available for amplifying sequences from a broad range of insects. Within these clusters, internal transcriber spacer regions (ITS1, ITS2) have been shown to reveal useful differences in length and sequence between closely related species (e.g., Black et al., 1989; Chen et al., 1999; Proft et al., 1999; Taylor & Szalanski, 1999). Amornsak et al. (1998) used PCR to amplify the ITS2 regions of the moth pests *Helicoverpa armigera* and *H. punctigera*, and a parasitoid of their eggs, *Trichogramma australicum*. The DNA sequence data obtained from the wasp were then used to create primers that permitted the specific amplification of *T. australicum* DNA from parasitised moth eggs. It was possible to detect parasitism only 12 h after the eggs were parasitised, and the authors considered that the method is likely to prove useful for evaluating the potential of *T. australicum* for biocontrol of *Helicoverpa* species in Australia. Zhu and Greenstone (1999) and Zhu et al. (2000) also used ITS2 regions to distinguish between *Aphelinus albipodus*, *A. varipes*, *A. hordei*, *A. asychis* and *Aphidius colemani*. DNA of *A. asychis*, *A. hordei* and *Aphidius colemani* was detectable in the Russian wheat aphid, *Diuraphis noxia*, as soon as one day after parasitism. Techniques have improved to the point where DNA from parasitoid eggs within host can be detected immediately after parasitism has taken place (Traugott & Symondson, 2008).

Other potential targets for molecular detection of parasitism are the 12 and 16S ribosomal RNA genes (rRNA) within mitochondria. With many hundreds of copies per cell, mitochondrial genes, again, potentially provide a large target for PCR. The rRNA genes are known to expose relatively recent evolutionary events (Hillis & Dixon, 1991), and have been shown to be effective at revealing differences between closely related species of Hymenoptera (Whitfield & Cameron, 1998). Most primers used for detecting parasitoids and other invertebrates are now based upon parts of the barcoding region of the mitochondrial COI gene (Folmer et al., 1994; Hebert et al., 2003; Hrček & Godfray, 2014; Frost et al., 2016). This region is conveniently conserved at, or close to, the species level.

Although a PCR stage increases the chance of detection by amplifying the target sequence, other approaches have been used successfully. Clearly, if PCR can be omitted, this could potentially increase the rate at which hosts might be screened. Greenstone and Edwards (1998) developed a species-specific hybridisation probe to a multiple-copy genomic DNA sequence from the braconid wasp *Microplitis croceipes*, and used it to detect parasitism of *Helicoverpa zea*. Despite the lack of a PCR stage, it could detect first-and second-instar larvae as efficiently as host dissection but was not effective for detecting parasitoid eggs. The probe reacted with the congener *M. demolitor*, but not with a representative growth stage of another parasitoid of this host (*Cotesia marginiventris*), or with *H. zea* itself. The authors reported that the whole procedure from host collection to evaluation of results is possible in around 24 h.

DNA-based approaches have now been developed that can be used to screen whole parasitoid communities attacking a target host. This can include both primary parasitoids and hyperparasitoids, as well as multiparasitism by different primary parasitoids species within the same host individuals (Traugott et al., 2008). This was achieved in a study of the parasitoid community attacking the aphid *Sitobion avenae* in wheat. In some cases, it was even possible to detect DNA from the remains of primary parasitoids attacked by the larvae of hyperparasitoids within a host. Such molecular approaches can rapidly screen large numbers of hosts and reveal

a lot about the ecology of the interactions involved. However, only rearing can tell us which parasitoid species might eventually emerge where multiparasitism is involved. In the Traugott et al. (2008) study, multiparasitism by primary hosts was found in 1.6% of the 1061 aphids screened.

An additional element facilitated by DNA-based approaches is that they may allow plant–herbivore–parasitoid–hyperparasitoid food webs to be extended to intraguild competitors, specifically predators (Moreno-Ripoll et al., 2012; Traugott et al., 2012). When predatory beetles or spiders attack aphids, for example, they are also consuming parasitoids within some of those hosts. The question then arises: do predators have a preference for parasitised over unparasitised hosts? There may be a nutritional advantage to doing so, or the predators may gain from eliminating intraguild competitors. Traugott et al. (2012) showed high rates of direct and indirect intraguild predation on parasitoids in wheat fields by carabid beetles and linyphiid spiders using PCR. Direct predation was attributed to predators that contained parasitoid, but no aphid, DNA in their guts. 'Coincidental' was where aphid and parasitoid DNA were both detected in the same individual predator. However, as the latter may be the result of either predation on parasitised hosts, or consumption of both adult parasitoids and aphids in quick succession, interpretation is difficult. In another recent study, Ye et al. (2017) were able to extend the food web to include herbivore (aphid)–parasitoid–hyperparasitoid–endosymbiont interactions.

Derocles et al. (2014), using molecular methods reported in Derocles et al. (2012), provide a good example of how host–parasitoid webs can now be constructed using molecular data. DNA extracted from aphids was targeted with group-specific primers designed to amplify the Aphidiinae. Fragments of two genes, the mitochondrial 16S and the nuclear LWRh, were amplified to refine parasitoid identification, in most cases to species level. When positive detection was found, the DNA was amplified and Sanger sequenced. Aphids from several different crops and from field margins were screened. Only three of the 32 parasitoid species observed were found in both the crops and the margins, indicating very little sharing of parasitoids and strong compartmentalisation in the food webs. The conclusion was that the field margins did not provide a source of parasitoids sufficient to suppress aphids within the crops and effect biocontrol.

There is continuing interest in the food webs that establish around invasive species. Again, molecular diagnostics can be used to detect and identify parasitoids within invasive hosts, providing rapid evidence regarding the species of parasitoid most likely to be capable of exercising some level of control. Recent examples include (Kitson et al., 2019) (parasitoids attacking the Oak Processionary Moth, *Thaumetopoea processionea*) and Gariepy et al. (2019) (parasitoids attacking the brown marmorated stink bug, *Halyomorpha halys*).

Detection of prey remains within predators.

DNA methods are also starting to be used in predation studies. There is an important role for such methods, which are, potentially, an efficient means of determining predator diet both qualitatively and quantitatively. The use of species-specific primer design, polymerase chain reaction (PCR) and most recently next-generation sequencing has greatly enhanced the use of molecular techniques to determine the role of predators in food webs (Symondson, 2002a, b; Sheppard & Harwood, 2005; Pompanon et al., 2012; Greenstone et al., 2014).

The sources of blood meals by haematophagous insects, such as mosquitoes, ticks and crab lice, have been detected using such techniques (Coulson et al., 1990; Tobolewski et al., 1992; Gokool et al., 1993; Lord et al., 1998; Gariepy et al., 2012). Prey DNA in the guts of predators and host-feeding parasitoids is likely to be in a highly degraded state, and so markers based on PCR techniques offer the most promise.

Zaidi et al. (1999) demonstrated that multiple-copy genomic DNA sequences that confer insecticide resistence on mosquitoes, *Culex quinquefasciatus*, could be detected for at least 28 h after the mosquitoes were fed to carabid

beetles, *Poecilus* (= *Pterostichus) cupreus*. Detection periods were shown to depend upon the length of DNA sequence, with successful amplifications of 146 and 263 bp sequences. Longer sequences could not be amplified reliably from gut samples, suggesting that short sequences survive intact for a longer period during the digestion process. The prey were equally detectable whether the beetles had eaten one mosquito or six, digested for zero or 28 h. DNA was extracted from the whole predator in this experiment, yet PCR was able to amplify the target sequences from the much smaller prey within the digestive systems of the carabid. Having demonstrated that the 40–50-fold replication of genomic multiple-copy genes allowed clear detection of semi-digested prey DNA within predators, Zaidi et al. (1999) speculated that the far greater copy number of mitochondrial genes per cell would make these attractive targets. This was confirmed by Chen et al. (2000), who used PCR primers to amplify mitochondrial COII sequences (77 to 386 bp) from six species of cereal aphid, and were able to detect aphid remains in the guts of predators. After feeding on a single *Rhopalosiphum maidis* aphid, then on five aphids of the related *R. padi*, a 198 bp fragment, specific for *R. maidis*, could be amplified from 50% of coccinellid predators after 4 h, and 50% of chrysopid predators after 9 h. The authors claim this technique to be superior to the use of monoclonal antibodies, in being more certain of success, faster, and less expensive to develop, yet of similar sensitivity and specificity. The overall costs of developing probes and assaying predators from the field appear to be comparable with those for ELISA.

Agustí et al. (1999) also found that detection periods were strongly affected by sequence length. Primers were designed to amplify sequence-characterised amplified regions (SCARs) derived from a randomly amplified polymorphic DNA (RAPD) band. Immediately after feeding ten eggs of the target prey, *Helicoverpa armigera*, to the predator, *Dicyphus tamaninii* (Heteroptera: Miridae), SCAR primers could amplify successfully 600 and 254 bp fragments, but not a larger 1100 bp sequence using a third set of primers. After four hours' digestion in *D. tamaninii*, only the 254 bp sequence could be detected in 45% of fed predators. In specificity tests, the primers failed to amplify a 254 band from any of the other species tested (five lepidopterans, two whiteflies and two predators), but, in two cases, did amplify sequences of different sizes. SCAR primers were also prepared from DNA of the glasshouse whitefly (*Trialeurodes vaporariorum*), and a short fragment (310 bp) was used to detect whitefly DNA in the gut of *Dicyphus tamaninii* (Agustí et al., 2000). At 25 °C, 60% of predators were positive four hours after eating ten *T. vaporariorum* nymphs, and the primer detected eggs and adults of this species, but also gave faint bands with other species of whitefly and aphid (Agustí et al., 2000). Hoogendoorn and Heimpel (2001) prepared four primer pairs, of different lengths, that were specific to the European corn borer, *Ostrinia nubilalis*. Using the shortest primer, a meal of corn borer eggs was detectable in the ladybird *Coleomegilla maculata* for up to 12 h, and longer primers gave shorter detection periods. The authors suggested that such a set of primers could be used to determine the range of times since feeding by predators in the field.

Attempts by Johanowicz and Hoy (1999) to detect 16S mitochondrial genes from *Wolbachia*, eaten by the mite predator *Amblyseius reductus* together with their host, the prey mite *Tetranychus urticae*, failed, probably because the chosen primers were designed to amplify a relatively long, 900 bp sequence. Greatorex (1996) prepared primers to detect segments of DNA of the prey mite *Orthotydeus caudatus* in its predator (the phytoseiid mite *Typhlodromus pyri*) using RAPD-PCR. Some primers amplified a range of mite species, but one pair were specific to *O. caudatus*, which it could detect in samples containing a 100,000th of a mite. The rate of digestion of prey DNA by the predator was not determined.

Future approaches could, potentially, be borrowed from studies of predation in vertebrate systems. PCR amplification of microsatellite markers (i.e., tandemly repeated short nucleotide

sequences) have been used successfully to detect predation in vertebrate systems. Scribner and Bowman (1998) used this method to identify species of waterfowl preyed upon by glaucous gulls. Some remnants of DNA from consumed goslings were estimated to be detectable 8–16 h after ingestion. Asahida et al. (1997) used PCR and restriction analysis of mitochondrial DNA to identify stone flounder (*Kareius bicoloratus*) DNA in the stomach of the sand shrimp (*Crangon affinis*), but for only up to five hours after ingestion. Their method can only detect the presence or absence of target DNA, but it has the potential for use in the simultaneous detection of multiple prey species. Other examples of detection of vertebrate prey remains in predator guts or faeces have been reviewed in Symondson (2002b), Sheppard and Harwood (2005), Pompanon et al. (2012).

There are now many reports of the detection of predation on target invertebrate species (usually a pest) in agricultural crops using one or more prey-specific primer pairs. This is a quick and simple approach, depending for its success mainly on the specificity of the primers. Recent examples include primers specific for pests such as the squash bug *Anasa tristis* (Schmidt et al., 2014), corn rootworm *Diabrotica vergifera* (Lundgren & Fergen, 2014), cassava whitefly *Aleurotrachelus socialis* (Lungren et al., 2014), coffee berry borer *Hypothenemus hampei* (Jaramillo et al., 2010), aphids (Chen et al., 2000) and leafhoppers (Virant-Doberlet et al., 2011). If a high proportion of the gut samples of predator contain DNA from the target species it is assumed that the predator might be capable of controlling pest numbers. However, this approach is being replaced to some extent by the advent of high-throughput (next-generation) sequencing (NGS). What a predator eats depends upon what is available. Alternative prey may divert the predators from eating the pests (reducing their pest controlling effect) or help to balance the nutritional requirements of the predator, leading to increases in the population density of that predator. There may be intraguild predation taking place, reducing the effectiveness of the predator community to control the pests. The advantage of NGS is that it can detect the whole range of invertebrate prey consumed. Food web analyses (Sect. 6.3.12) can then be used to determine the strengths of the interactions taking place. Software has been developed (e.g., the 'econullnetr' package in R statistical software) that allows the relative densities of different prey in the environment (determined by a survey) to be compared with the frequency with which different prey are consumed (measured by NGS detecting numbers of predators testing positive for each prey) (Vaughan et al., 2018). Essentially the approach tests for deviations from random feeding, which can show either preference or avoidance. Pearson et al. (2018) provide an example of using this approach in an aquatic system. In this study, feeding by two competing predator species (the Caddisfly *Rhyacophila dorsalis* and the Stonefly *Dinocras cephalotes*) was analysed in a community of 30 prey species. The presence of key species within rivers is widely used to measure its health, but to date there has been little regard paid to the trophic relationships that may affect species abundance, especially in relation to agricultural intensification gradients. Few studies have used NGS to analyse predation by invertebrates in other applied contexts, such as agriculture, horticulture and forestry. In more ecological contexts, NGS has been used to analyse the diets of spiders in a range of habitats, including high arctic, sea shore, forest and desert environments (e.g., Petráková et al., 2015; Hambäck et al., 2016; Toju & Baba, 2018; Eitzinger et al., 2019). However, linyphiid spiders have been analysed in a study of their diets in arable fields (Pinol et al., 2014).

When general invertebrate primers are used to analyse gut samples from invertebrates, the expectation would be that most of the sequences detected derive from the predator, rather than from the degraded prey in its gut. While it is possible to partially prevent this by using blocking probes (Vestheim & Jarman, 2008), such probes may block the detection of some prey (e.g., a spider predator eating other spider species). By not using a blocking probe, Pinol et al. (2014) found that although most of the sequences obtained were from the spider predator (*Oedothorax fuscus*),

there was sufficient depth to the sequencing to show that this cereal crop spider had been eating members of the Diptera, Lepidoptera, Collembola and Nematoda, as well as other spider species. The numbers of prey sequences that can be amplified from spiders can be maximised, while minimising amplification of the predator sequences, by selectively removing longer amplicons of DNA and only extracting from DNA from the mid and hind gut (Krehenwinkel et al., 2017): long-amplicon DNA is more likely to derive from the predator while short-amplicon DNA will predominantly derive from semi-digested prey remains. Pearson et al. (2018) found that if DNA was extracted from the gut contents by dissection first, rather than extracting DNA from the whole homogenised predator or segments thereof, minimal amplification of the predator resulted when no blocking probe was applied.

It is also possible to combine the use of species-specific primers with NGS. For example, Gomez-Polo et al. (2016) used specific primers to analyse predation by *Orius majusculus* on aphids (*Nasonovia ribisnigri*) and thrips (*Frankliniella occidentalis*) in a lettuce crop, then applied NGS with general primers to detect predation on other invertebrates, including intraguild competitors: Collembola proved to be a major alternative prey. Similar methods were used to analyse the diets of hover flies (Syrphidae) in the same crop (Gomez-Polo et al., 2015).

A different approach used in some studies has been to design separate primers for each target prey species and develop multiplexes allowing many species to be analysed at the same time. This was applied to analyse predation on aphids, alternative prey and intraguild predators in cereal fields (Staudacher et al., 2016, 2017; Roubinet et al., 2017). The advantage of this approach is that it can precisely target species known to be present within the crop. The advantage of NGS is that it should, in principle, amplify all prey species, without prior knowledge of what is present. In practice, no general primers have yet been developed that amplify every invertebrate species and therefore more than one set of general primers, with abilities to amplify different ranges of taxa, may need to be applied, adding to costs.

Many vertebrate predators eat invertebrates and NGS has been used to detect predation on crop pest species as well as alternative prey. For example, Taylor et al. (2017) demonstrated that a third of faecal samples from bats contained the DNA of the stink bug *Nezara viridula*, a major pest of macademia. Other bats have been shown, for example, to consume the pine pest *Bupalus pinaria* (Krüger et al., 2014), pests of oil palm plantations (Brandon-Mong et al., 2018), mosquitoes (*Culex pipens*), black flies (*Simulum* spp.) and paper wasps (*Polistes* spp.) (Clare et al., 2014). Similarly, NGS has been used to show that birds, such as Western Bluebird, (*Sialia mexicana*), are potentially valuable predators of pests in vinyards, consuming herbivorous Hemiptera and Lepidoptera, as well as mosquitoes (Jedlicka et al., 2017).

6.3.12 Food Web Construction and Analysis

The methods described above can help both to determine and to quantify predator–prey and parasitoid–host relationships. However, to appreciate fully the significance of these relationships for the organisation and functioning of a community, techniques for integrating and analysing these data are required. This is a non-trival task because understanding ecological communities is among the most complex challenges in any scientific discipline (Godfray & May, 2014). This is due to the large number of organisms and inter-organism interactions involved, each with their own evolutionary and co-evolutionary histories (Peralta et al., 2014; Levine et al., 2017). It is also a desirable task because it allows assessment and even prediction of how communities respond to natural and anthropomorphic impacts (Montoya et al., 2015; Rocca & Greco, 2015; Sanders et al., 2018).

Food webs (trophic webs) can be used to map all the trophic interrelationships of interest in a particular system (Bukovinszky et al., 2008; Peralta et al., 2014; Montoya et al., 2015; Henri & van Veen, 2016; Sanders et al., 2018), in principle considering both species diversity and

the transfer of metabolites between species through their interactions (Montoya et al., 2015). Mapping trophic interrelationships of interest in a particular system gives a more or less static representation of a community (Hall & Raffaelli, 1993), and manipulation experiments may be preferable for investigating the dynamics of community organisation (Memmott et al., 1994; Frost et al., 2016; Barbosa et al., 2017). Unfortunately, for many communities, it is not feasible to manipulate component species on the scale required, and, in these cases, the detection of patterns in food webs is the next best means of gaining insights into community structure and function. Methods to describe and analyse the more complex and dynamic aspects of food webs, including the many categories of potential indirect effects, are still being developed and debated (Abrams et al., 1996; Polis et al., 1996; Blüthgen, 2010; Poulin, 2010; Fontaine et al., 2011; Allesina & Tang, 2012).

Host–parasitoid trophic relationships are more easily quantified than is the case for predators and their prey (Godfray & Müller, 1999; Lewis et al., 2002) and construction of trophic webs has been successful in the study of communities of insect herbivores and their parasitoids (Memmott & Godfray, 1994; van Veen et al., 2006a, 2006b, 2008; Bukovinszky et al., 2008; Hrček & Godfray, 2014; Peralta et al., 2014; Maunsell et al., 2015; Rocca & Greco, 2015; Henri & van Veen, 2016; Sanders et al., 2018). In contast, dietary information for predators is usually incomplete, and often reflects the amount of observational effort expended (e.g., the number of prey species of the scorpion *Paruroctonus mesaensis* reached 100 on the 181st survey night, and never reached an asymptote after 2000 person-hours of observation over five years, Polis, 1991). DNA-based molecular methods have recently revolutionised the elucidation of food webs, revealing trophic links that otherwise would not be detectable with standard rearing methodologies (e.g., Condon et al., 2014; Hrček & Godfray, 2014; Wirta et al., 2014; Frost et al., 2016).

There are a number of different types of food web (Memmott & Godfray, 1994). Some show trophic linkages only, while others also describe the strengths of these interactions, but note that webs typically include little or no information on the factors that determine the linkages themselves:

1. ‘Connectance web’—only the existence of interactions between host/prey and natural enemy species is recorded (i.e., a topological food web; Shameer et al., 2018; Woodward & Hildrew, 2001; Yodzis & Winemiller, 1999) (Fig. 6.26).
2. ‘Semi-quantitative web’—the relative numbers of (or % parasitism by) each natural enemy species per host/prey species are recorded (e.g., Dawah et al., 1995).
3. ‘Quantitative web’—the densities of host/prey, natural enemies and the strengths (i.e., % parasitism) of host/prey–natural enemy interactions are recorded (e.g., Müller et al., 1999; Schönrogge & Crawley, 2000; Lewis et al., 2002; Hirao & Murakami, 2008; Peralta et al., 2014; Wirta et al., 2014; Maunsell et al., 2015; Rocca & Greco, 2015; Barbosa et al., 2017; Sanders et al., 2018) (Fig. 6.26).
4. ‘Source web’—is centred on a plant or herbivore and extends to all higher trophic levels (e.g., Hövemeyer, 1995; Memmott et al., 2000).
5. ‘Sink web’—is centred on a natural enemy and includes all relevant lower trophic levels (e.g., Schoenly et al., 1991).
6. ‘Community web’—is non-centred and includes all species (e.g., Schoenly et al., 1991).

Webs can also be site specific, time specific (e.g., for a single crop growth stage), or cumulative (gathered over many occasions within a discrete area). The IRRI cumulative rice web, for example, contains 687 taxa and more than 10,000 trophic links (Schoenly et al., 1996a). Some published webs combine data from localities, seasons or years, (‘composite webs’, e.g.,

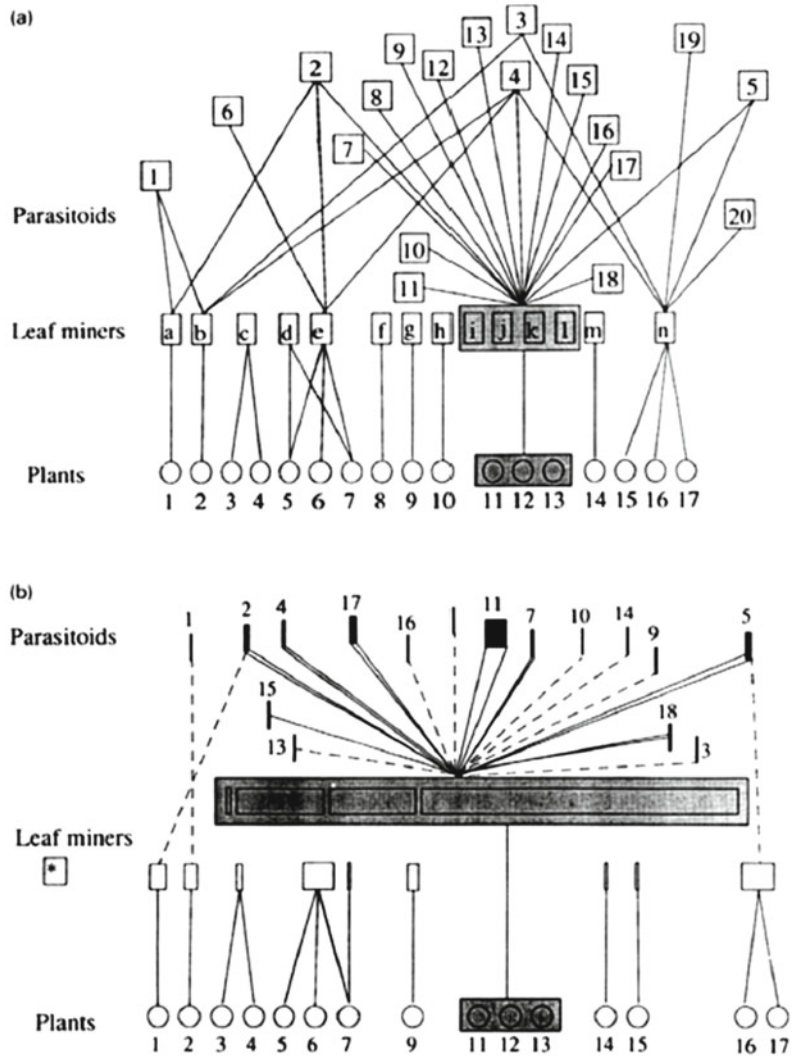

Fig. 6.26 Comparison of a connectance web and a quantified web for a plant–hispine–parasitoid food web. **a** Connectance web displaying all interactions, **b** quantified web showing the absolute abundance of leafminers, parasitoids and interaction strength. The first rectangle (*) in the leafminer level represents 30 miners-parasitoids reared per 1000 m^2. Numbers and letters each refer to different species of plant, leafminer and parasitoid. *Source* Memmott and Godfray (1993). Reproduced by permission of CAB Publishing

Dawah et al., 1995; Shameer et al., 2018, or 'metawebs', Frost et al., 2016), but there is a danger that this may obscure subtle patterns in web structure. Constructing webs for the overall community and separately for each site and season allows assessment of the potential for spatial and seasonal variability in the distribution of feeding links to affect community stability (Henri & van Veen, 2016). For instance, studying both pest infestation and non-infestation periods can help to assess the importance of intercrops for the maintenance of lepidopteran and parasitoid populations in cropping systems (Saeed et al., 2015; Shameer et al., 2018), and comparing food webs constructed within each period would likely be informative.

Web studies vary in the degree of their trophic resolution (e.g., in some studies facultative hyperparasitoids are not distinguished from other parasitioids, Lewis et al., 2002). Webs may be constructed from either taxonomic species or trophic species (i.e., functional groups that contain organisms that eat and are eaten by the same species within a food web, Cohen & Briand, 1984), and less sampling effort is required to characterise webs accurately in the latter case (Martinez et al., 1999). Trophic species (= trophospecies) are currently delimited

subjectively, and it would be better to use objective quantitative methods, but the identification of optimal methods to do so is proving to be elusive (Yodzis & Winemiller, 1999).

Web data are useful in a variety of contexts (Memmott & Godfray, 1994), such as: (a) comparison between hosts in different feeding locations, (b) comparison between early- and late-successional habitats, (c) comparison between tropical and temperate regions, (d) comparsion between low and high elevation (Maunsell et al., 2015), (e) study of the determinants of parasitoid host range, (f) as a guide to the probability of apparent competition (Holt & Hochberg, 2001; van Veen et al., 2006b, Sect. 7.3.7) between hosts (Rott & Godfray, 2000; Lewis et al., 2002; Frost et al., 2016; Shameer et al., 2018), (g) to probe the intricacies of community structure and function (e.g., degree of compartmentation, Montoya et al., 2015; influence of keystone species, role of the frequency distribution of different body sizes, Woodward & Hildrew, 2002), (h) to assess the effect of alien hosts on native parasitoids and native hosts (Schönrogge & Crawley, 2000), (i) to quantify prenetration of food webs in native habitats by introduced alien parasitoids (Henneman & Memmott, 2002), (j) to assesss host–parasitoid communities establishing in introduced crops (Rocca & Greco, 2015), and (k) to evaluate community stability and vulnerability to extinction (Peralta et al., 2014; Barbosa et al., 2017; Sanders et al., 2018). Comparison of collections of food webs may uncover patterns in the structure of natural communities (Rott & Godfray, 2000). Study of food webs is also likely to guide the development of models into profitable and realistic directions (Cohen et al., 1994; Memmott & Godfray, 1994).

Food web data can also be valuable in underpinning other studies. West et al. (1998), for example, used knowledge of leafminer–parasitoid and aphid–parasitoid–hyperparasitoid food webs in an investigation of the potential mechanisms of horizontal transfer of various strains of *Wolbachia* bacteria, which can cause parthenogenesis in parasitoids (Sect. 6.5). Valladares and Salvo (1999) compared plant—leafminer (Diptera: Agromyzidae)—parasitoid webs in a natural area (woodland and grassland), and an agricultural area (containing various crops, including potatoes, brassicas and beans). They considered that exercises of this sort could be useful in identifying parasitoids with potential for the biological control of pests.

There are now many, often closely interrelated, metrics developed for studying food webs (Memmott & Godfray, 1993; Peralta et al., 2014; Maunsell et al., 2015; Montoya et al., 2015; Frost et al., 2016; Barbosa et al., 2017). These can facilitate comparisons between webs (Schmid-Araya et al., 2002) and can indicate community productivity (Montoya et al., 2015). Further, descriptive metrics can be fitted as response or exaplanatory variables (Chap. 9) within formal null hypothesis testing statistical analyses of food web properties (Peralta et al., 2014; Maunsell et al., 2015; Montoya et al., 2015; Frost et al., 2016; Barbosa et al., 2017).

Such metrics include: (a) number of species [S] and trophic levels, number of links (trophic interactions) [L], (b) linkage density or 'average degree' [L/S], (c) connectance, a proportional measure of community complexity, usually the number of trophic interactions divided by the number of possible interactions [L/S^2] (e.g., Warren, 1994; Maunsell et al., 2015; Montoya et al., 2015; Rocca & Greco, 2015, reviews various definitions of connectance), (d) average interaction strength between species (Laska & Wootton, 1998, identify four different theoretical concepts of interaction strength), (e) ratios of number of species at different trophic levels, (f) degree of compartmentation (i.e., where species interactions are arranged in blocks and within-block interactions are strong but between-block interactions are weaker, Raffaelli & Hall, 1992; Montoya et al., 2015), (g) number of species feeding at more than one trophic level (omnivory), (h) incidence of trophic loops (e.g., A attacks B attacks A, Polis et al., 1989), (i) overlap, the number of pairs of species at one trophic level that share at least one natural enemy at the next highest trophic level, divided by the total possible number of such links (van Veen et al., 2008; Frost et al., 2016), (j) redundancy, the average number of natural enemies attacking

each host species weighted by the frequency of the trophic interactions (Peralta et al., 2014), redundancy is high when many species feed on the same resource in the same way, and (k) trophic complimentarity, a measure related to overlap and nestedness, describing how connected a food web is (Peralta et al., 2014; Montoya et al., 2015): species specialising by feeding on different resources leads to high resource complimentarity and species having different natural enemies leads to high predation complimentarity, with trophic complimentarity representing the interaction between these.

Some of these metrics are sensitive to the degree of taxonomic and trophic resolution of the web, but the majority are stable over a wide resolution range (Sugihara et al., 1997). Various types of robustness analysis can be used to quantify the extent to which sampling effort may bias these web metrics. Lewis et al. (2002), for example, investigated the effects of omitting the least frequent linkages from the dataset (84 parasitoid species attacking 93 species of leafmining insect in tropical forest in Belize). They concluded that their sampling programme had uncovered the majority of host and parasitoid species in the community under study, but not all the interactions among species. Quantitative webs can give strong indications about which species are likely to be keystone species (e.g., species whose interactions dominate the system numerically), and may also be useful for testing the current hypotheses of food web theory (Müller et al., 1999). Not all web types are suitable for estimating the general properties of community webs. Hawkins and Marino (1997), for example, showed that webs based on single, or few, sources underestimated the number of links per species, compared with full webs, and produced inconsistent estimates of the ratios of number of species at the different trophic levels.

Practical problems to be aware of when constructing a web include: (a) the difficulty of making large collections of uncommon hosts for rearing (Schönrogge & Crawley, 2000), a limitation for a connectance web, but less serious for a quantitative web (where interactions between rare prey/hosts and their natural enemies do not have a strong numerical influence); (b) sample bias in relation to parasitised versus unparasitised hosts, and between parasitoid species that take very different times to develop in the host, or bias against collecting parasitoids that attack very young or very small hosts; (c) bias due to differential ease of rearing-out (Rott & Godfray, 2000; Lewis et al., 2002); and (d) failure to sample unexpected host locations (e.g., leafminers pupating on the ground) (Memmott & Godfray, 1994).

Some of these problems can be overcome by following the suggestions given in Sects. 6.3.5 and 6.3.6. Müller et al. (1999) stress the importance of obtaining quantitative information specifically for food web construction, rather than adapting data that was previously collected for some other purpose. This directed approach also means that data collection can employ common methodologies, shared between different communities, which will then facilitate more meaningful comparisons between these communities. Some progress may nethertheless be made, in the absence of such quality information, at least to provide a tentative estimate of community structure and to suggest directions for future, more directed, studies. For instance, Shameer et al. (2018) constructed continental-scale connectance webs of the 'plant–lepidopteran herbivore–parasitoid' community in coconut agro-ecosystems using literature records for the Indian sub-continent. They found that that crop and intercrop plant species shared few herbivore pest species, suggesting that feeding herbivores are unlikely to experience direct interspecific competition, a promoter of competitive exclusion. They also found that pest herbivores share large proportions of their parasitoids, suggesting that indirect ecological interactions (apparent competition, Holt & Lawton, 1994; Sect. 7.3.7) between herbivore species are likely to be important determinants of herbivore and parasitoid population dynamics (Shameer et al., 2018).

It should be noted that detection of some of the rarer interactions in a web will only become apparent after very intensive and extensive surveying. For example, Dawah et al. (1995)

demonstrated the existence of tertiary parasitism in grassland galler-parasitoid communities in the UK, but parasitoids of hyperparasitoids were recorded for only 63 out of 64,781 observations. Woodward and Hildrew (2001) showed that, in food web studies involving predation, a large number of gut contents samples are needed to achieve an acceptable degree of accuracy. They constructed yield-effort curves for the dominant predators in their study system (an acid stream flowing through a lowland heath) to examine how the cumulative percentage of feeding links detected increased with cumulative number of guts examined. Only species that had reached an asymptote for their feeding links were included in the calculation of food web statistics, and, in most cases, this required several hundred gut contents samples per species.

There are many methodological variants that have been employed in the construction of different types of food web, for various natural enemies and communities. The study of Müller et al. (1999), however, will be described as an example of some procedures for sampling and analysis that can be very useful. These authors constructed monthly quantitative parasitoid webs for aphid–parasitoid relationships (based on 71 species of aphid and parasitoid) in an abandoned damp field in England over a period of two years (Godfray & Müller, 1999). Random plant units (usually a whole plant or a grass ramet) in each square of a 20 × 20 m grid were taken twice each month, and the numbers of aphids and mummies on these were counted. Aphid and mummy densities were calculated after measuring the number of plant units per m^2. Mummies were placed in gelatin capsules until the parasitoids emerged. Monthly webs were visualised in diagrams (Fig. 6.27) showing the relative abundance (indicated by bar width) of different aphid species in the centre of the diagram, with the relative abundance of primary parasitoids below, and the relative abundance of secondary parasitoids above that of the aphids, and with wedges

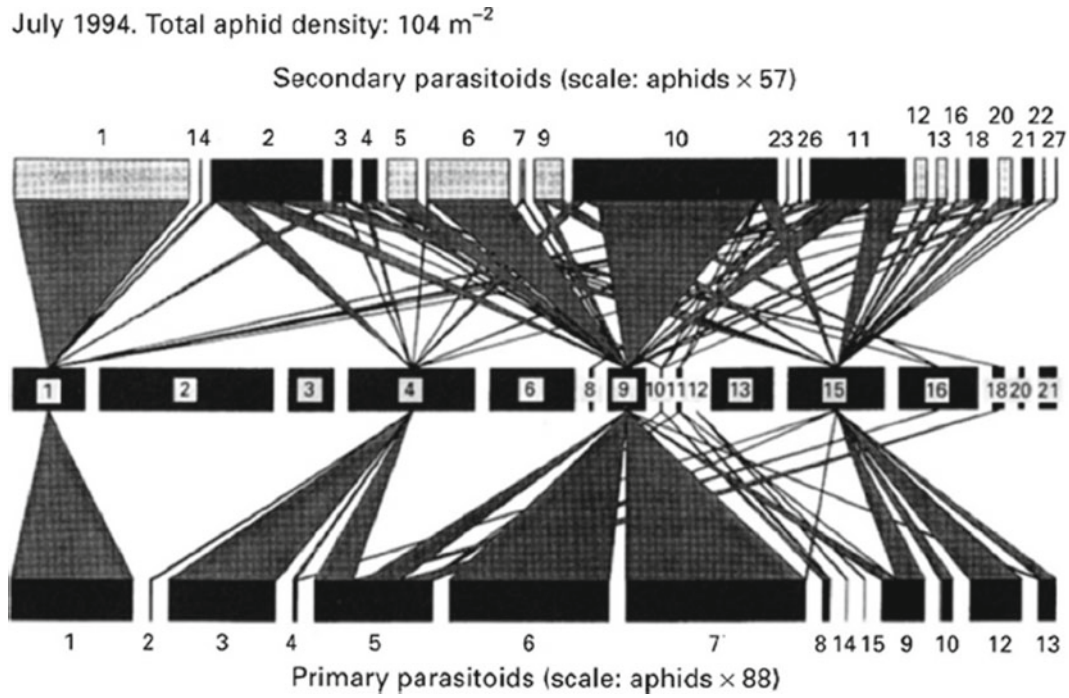

Fig. 6.27 Example of an aphid–parasitoid web in an abandoned field in southern England, July 1994. Relative aphid abundances are shown in the centre with primary parasitoids below and secondary parasitoids above (hyperparasitoids in grey and mummy parasitoids in black). Each number refers to a different species. Species densities are shown to scale. *Source* Müller et al., (1999). Reproduced by permission of Blackwell Publishing

indicating the relationships between species. These illustrations were produced with low-level graphics routines in the computer package Mathematica (more recent and freely available software [for Microsoft Windows platforms] for visualising interaction networks is provided by Sint & Traugott, 2016). The Dirichlet distribution (Goodhardt et al., 1984) was found to be an effective means of summarising species compositions in the different webs, and showed that there were many rare, and few common, species of both aphids and parasitoids. Parasitoid overlap graphs (Fig. 6.28) were constructed to summarise the patterns of shared parasitism. In these graphs, vertices, representing different aphid species, are linked by lines if both are attacked by a species of the same category of parasitoid (i.e., primary parasitoids, hyperparasitoids that attack the primary parasitoid within the living aphid, and mummy parasitoids that attack the mummy irrespective of whether it contains a primary or a secondary parasitoid). These graphs illustrate the potential for aphid species on different host plants to interact indirectly through shared parasitoids (apparent competition, Holt & Lawton, 1994; Frost et al., 2016; Chap. 7), so they provide a simple visual indication of the potential cohesiveness of the community. In speciose communities, it may be more practictable to illustrate the same information in tabular form (Shameer et al., 2018).

Polyphagous parasitoids may exert more influence on a given host species if alternative host species are present in the habitat (Lawton, 1986; Settle & Wilson, 1990), and parasitoid overlap graphs summarise the extent to which diverse host reservoirs are available to this category of parasitoid. Müller et al. (1999) developed a new type of graph (the quantitative parasitoid overlap diagram) to show the relative importance of any one species of aphid as a source of parasitoids to attack other species of aphid (Fig. 6.29). In these diagrams, which are modifications of the traditional parasitoid overlap graph, the size of each vertex circle (again representing an aphid species) is proportional to aphid abundance, and the degree of vertex shading and the width of the links are related to the degree to which each aphid species is a source of parasitoids attacking itself or other aphid species. These diagrams were useful in the Müller et al. (1999) study, in showing that aphid-primary parasitoid dynamics of the common aphid species were not strongly linked, whereas mummy parasitoids attacking the common aphid species were not dominated by individuals that had developed in those common species (i.e., few aphid species acted as the main source of their own mummy parasitoids). Mummy parasitoids were thus shown to be important agents of linkage in the community between distinct aphid–parasitoid–hyperparasitoid sets. This method was extended to examine apparent competition in food webs determined with DNA-based methods in Frost et al. (2016). Hrček and Godfray (2014) provide an overview of the potential of molecular genetic methods in food web research, and a detailed analysis of how molecular data may be turned into ecological networks can be found in Clare et al. (2019). Construction of food webs based upon molecular detection of parasitoids within hosts and prey within predators is discussed in Sect. 6.3.11.

Quantitative parasitoid overlap diagrams have also been used to display the extent to which leafminer hosts (twelve species of *Phyllonorycter* attacking alder, willow, oak and beech) are a source of their own parasitoids, or a source of parasitoids attacking congeners (Rott & Godfray, 2000), and to show the potential for apparent competition between lepidopterans in native and planation forests (Frost et al., 2016). As with non-quantative overlap graphs, quantitative parasitoid overlap can be presented in tabular form and it may further be informative to calculate overlap separately for differnet classes of natural enemy, such as egg, larval and pupal parasitoids (Shameer et al., 2018).

Graphs of predator overlap (links joining predators that share prey) and prey overlap (links joining prey that share predators) can also be constructed. In a study of changes in a stream food web after invasion by a new top predator (the dragonfly *Cordulegaster boltonii*), Woodward and Hildrew (2001) found that pre- and post-invasion predator overlap graphs could be

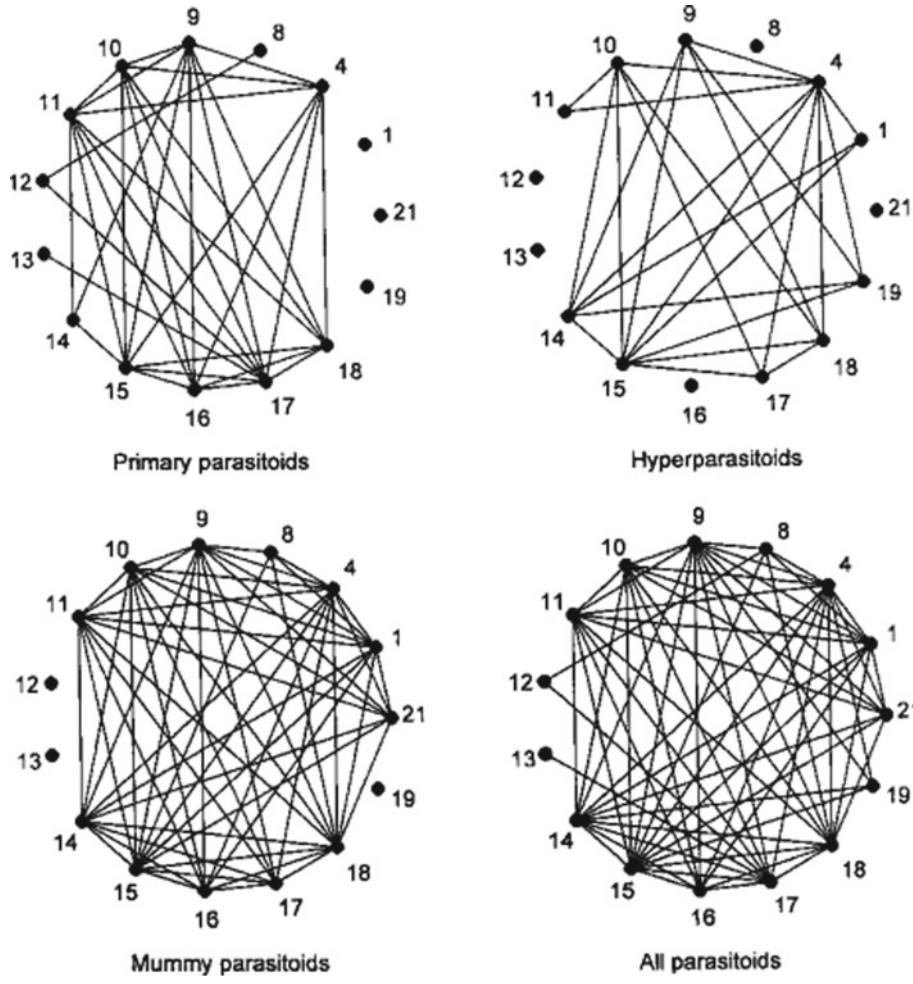

Fig. 6.28 Parasitoid overlap graphs for an aphid–parasitoid web in an abandoned field in southern England. The vertices represent the aphids from which at least one species of parasitoid were obtained. Vertices are joined by edges when the aphids share at least one species of parasitoid. Each number refers to a different species. *Source* Müller et al., (1999). Reproduced by permission of Blackwell Publishing

represented in one dimension (termed 'interval graphs'), but that the prey overlap graphs were non-interval because several species could not be placed in one dimension with other species in the graph. Interval graphs denote a high degree of interconnectedness (in this case a high degree of generalist feeding), which non-linear modelling suggests may stabilise food webs (McCann et al., 1998). In the acid fish-free stream studied by Woodward and Hildrew (2002), all six invertebrate predators ate nearly every invertebrate taxon smaller than themselves. To summarise this system, and its dynamic changes, they presented a series of monthly intraguild food webs and niche overlap webs (the latter based on Pianka's Niche Overlap Index, Pianka, 1973). In these web diagrams, circles (representing predator species) varied in area according to predator density, and vertical location of circles was used to indicate the relative body size of each predator species. Intraguild predation in this system was shown to be strongly asymmetric (with larger predators dominant) and niche overlap decreased as discrepancy in body size increased.

In spite of the importance of host–parasitoid web analyses in themselves, it remains desirable

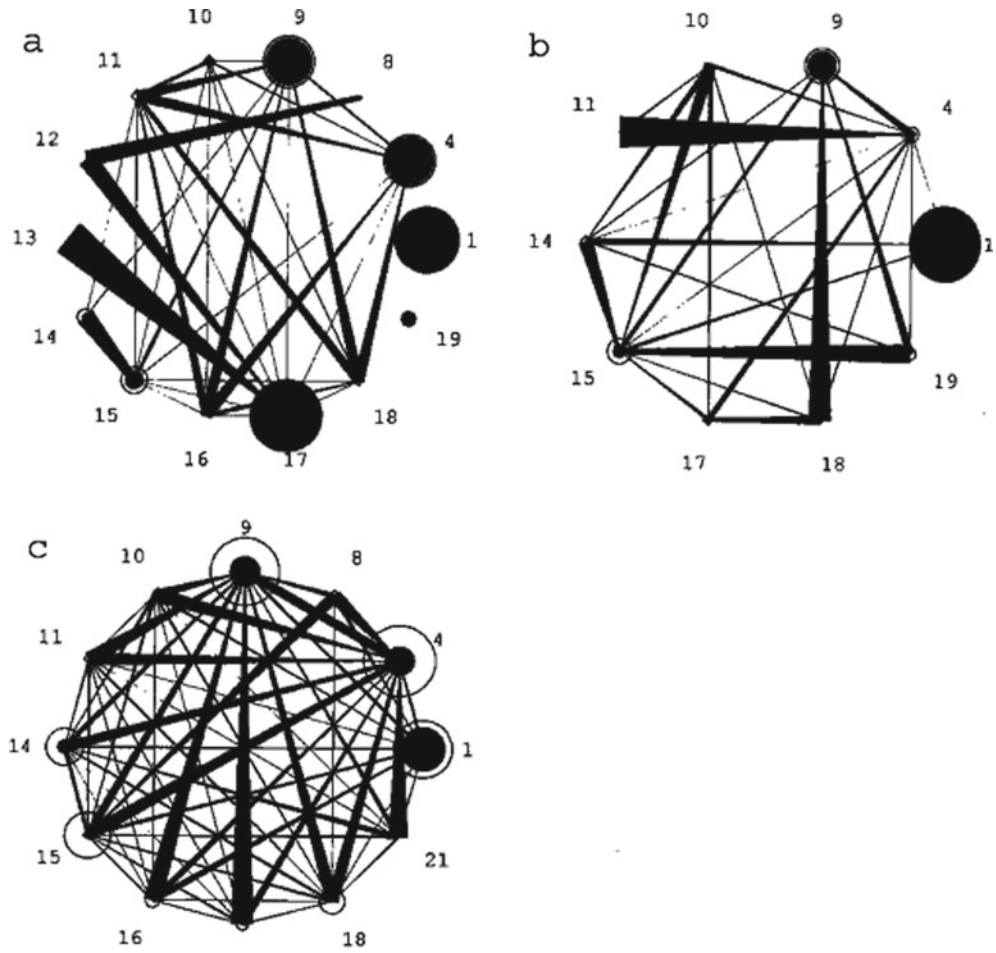

Fig. 6.29 Quantitative parasitoid overlap diagrams for an aphid–parasitoid web in an abandoned field in southern England. The vertices represent aphids and they are linked if the two species share a parasitoid. The size of each vertex is proportional to aphid abundance. The extent to which the vertices are coloured black, and the strength and symmetry of the links, measure the importance of each aphid species as a source of parasitoids attacking other species of aphids and itself. Each number refers to a different species: **a** primary parasitoids, **b** hyperparasitoids, and **c** mummy parasitoids. *Source* Müller et al., (1999). Reproduced by permission of Blackwell Publishing

to integrate further information about predators and pathogens that attack the same species of host (Godfray & Müller, 1999). Van Veen et al. (2008) conducted one of the first of these studies, creating a food web including 29 aphid, 24 parasitoid, and 13 predator species. Interactions between these natural enemy groups (Sunderland et al., 1997) could also influence community dynamics. In general, over-simplification of food web descriptions in the past may have misled theorists into making erroneous assumptions and generalisations (Polis, 1991; Hall & Raffaelli, 1993).

Quantifying the degree of compartmentation of food webs can be a useful step towards understanding their dynamics. A keystone predator, for example, can maintain a diversity of prey species by preventing a single dominant prey species from out-competing a range of other prey species, which form the base of a whole compartment of prey, predator and hyperpredator species. Thus, knowledge of compartmentation

enables prediction of cascade effects arising from removal or inhibition of keystone predator species (Raffaelli & Hall, 1992). Raffaelli and Hall calculated the trophic similarity between all species in a web using the Jaccard coefficient, $S_{ij} = a/a + b + c$ (where a is the number of species that interact with i and j, b is the number of species interacting with i only, and c is the number of species interacting with j only), and then graphed the frequency distribution of these coefficients. Webs with no compartments exhibit more or less unimodal distributions of trophic similarity, whereas for compartmented webs the distributions will be U-shaped, bi- or polymodal. They then carried out an ordination analysis (Hill, 1973) on the similarity matrix, to identify which species comprised the compartment. Compartmentalisation (or modularity) can be informative in a biocontrol context, in which it may demonstrate the degree to which natural enemies are shared between crop and non-crop habitats. For example, both Macfadyen et al. (2011), Derocles et al. (2014) showed little sharing of parasitoids, suggesting that non-crop areas may not be as useful as is usually assumed as sources for at least some groups of natural enemies.

Food web methodology can be profitably applied in ecotoxicological investigations, as a powerful summary of system changes following pesticide applications. Schoenly et al. (1996b) constructed plot-specific food webs for a plot of irrigated rice in the Philippines, sprayed with the synthetic pyrethroid deltamethrin, compared with an unsprayed control plot. Species composition and abundance were determined from vacuum netting (Sect. 6.2.2), and trophic relationships were taken from the larger 'Philippines cumulative rice web', i.e., 546 taxa and 9319 consumer-resource links at 23 sites (Cohen et al., 1994). Schoenly et al. (1996b) calculated the magnitude and direction of differences in percentage herbivores, and natural enemies of herbivores, on fourteen sampling dates, and the mean food chain length (mean number of links of maximal food chains from basal species to top predator), of sprayed and unsprayed webs for each date. The insecticide was found to cause a dramatic increase in percentage herbivores, and a reduction in mean food chain length. These changes were associated with pest outbreaks.

6.4 Genetic Variability in Field Populations of Natural Enemies

Individuals within and among populations of the same natural enemy species vary genetically (Hopper et al., 1993; Wajnberg, 2004, 2010; Chap. 3). Patterns of genetic variation within and among populations, usually assayed using molecular markers, reflect historical and contemporary processes such as mutation, selection, drift, and gene flow. These patterns of genetic variation can thus be used to infer information about dispersal (gene flow) over relatively small geographic areas, and geographic origins of natural enemies over larger distances (Lombaert et al., 2014; van Nouhuys, 2016). In addition, within-species genetic variation is often expressed as marked differences in phenotypic attributes of natural enemies such as morphology, physiology, and behaviour (Wajnberg, 2004, 2010; Lommen et al., 2017).

A variety of molecular markers can be used to quantify patterns of genetic variation within and among populations. The frequency of use of various molecular markers has changed dramatically over time (Schlötterer, 2004; Seeb et al., 2011). In general, molecular markers used to study within-species genetic variation can be divided into three conceptual classes (Schlötterer, 2004): (1) protein variants (allozymes), (2) DNA sequence polymorphisms (e.g., amplified fragment-length polymorphisms—AFLPs, restriction fragment-length polymorphisms—RFLPs, single nucleotide polymorphisms—SNPs, next-generation sequencing—NGS), and (3) DNA repeat variation (e.g., microsatellites). Allozymes, AFLPs, and RFLPs were commonly used in the past but have now been superseded by microsatellites, SNPs, and NGS (Schlötterer, 2004; Seeb et al., 2011). Single-locus, low-variability markers such as mitochondrial DNA, while useful for species-level identification, have also fallen out of favour as stand-alone tools for

studying within-species genetic variation (Allendorf, 2017). Advantages and disadvantages of different molecular markers, details of the analysis of molecular marker data, and the best applications for each type of marker are described elsewhere (Morin et al., 2004; Schlötterer, 2004; Ekblom & Galindo, 2011; Guichoux et al., 2011; Seeb et al., 2011; Putman & Carbone, 2014; Allendorf, 2017). In general, as the cost of generating DNA sequence data continues to decrease and the ease of sequence data processing increases, there is a progressive move towards the use of SNPs (Seeb et al., 2011), including the use of NGS techniques to generate genome-wide SNP data for natural enemies (e.g., Zhang et al., 2018a, b). However, the generation of large amounts of DNA sequence data is not necessary to answer all questions about genetic variation in natural enemy populations. For example, microsatellite markers are still widely used and are particularly useful for applications such as parentage analysis (Seeb et al., 2011). Van Nouhuys (2016) gives a concise overview of research using different molecular markers used to study genetic variation and population structure of parasitoids.

Most natural enemy populations are patchily distributed across landscapes, due to discontinuities in the distribution of hosts or prey, or other essential resources. The movement of individuals within and among local populations (within which there is regular movement of individuals between habitat patches), a series of which forms a metapopulation (a collection of local populations with relatively little movement of individuals among them), is reflected in the genetic structure of a given population; i.e., the degree of differentiation of local populations within a metapopulation (Couchoux et al., 2016). The amount of genetic structure within and among local populations is influenced by the dynamics of local populations, the structure of the landscape (e.g., barriers to or facilitators of dispersal), and the inherent dispersal ability of the natural enemy species in question (Kankare et al., 2005). Couchoux et al. (2016) used microsatellite markers to study the metapopulation structure of the parasitoid *Hyposoter horticola* in a fragmented island landscape. In contrast to its host butterfly, which has strong spatial population structure, the parasitoid had low spatial genetic structure due to a high dispersal ability. Estimated as the maximum geographic distance between full-sibling parasitoids (using microsatellite markers), the maximum dispersal range for the parasitoid was estimated to be 7.5 km. The reverse situation was described by Wei et al. (2017), again using microsatellite markers. They showed that, in China, the parasitoid *Cotesia vestalis* has higher levels of genetic differentiation (indicating less gene flow) among populations compared to its migratory host, *Plutella xylostella*, indicating that parasitoid populations do not engage in annual migrations with their host, but rather form resident local populations. Nair et al. (2016) used microsatellite markers to study the metapopulation dynamics of the fourth trophic level (a hyperparasitoid, *Mesochorus stigmaticus*). They found that the population genetic structure of the hyperparasitoid was weaker (indicating more gene flow across the landscape) than that of the primary parasitoid or the host. It was concluded that although resources may be more fragmented for higher trophic levels, they are sometimes able to overcome this fragmentation to some degree with high dispersal ability.

Studying the structure of genetic variation can also help to understand gene flow at larger spatial scales; for example, to reconstruct the history of the introduction of organisms to new continents. While these techniques have mostly been applied to invasive pest species to date (Estoup & Guillemaud, 2010), they are likely to be applied to natural enemies more in the future due to the large number of recent adventive (unintentional) introductions of natural enemies to new areas outside of their native range (Mason et al., 2017). A notable example of using genetic variation to reconstruct introduction pathways of a natural enemy comes from studies of the harlequin ladybird beetle, *Harmonia axyridis*. Analyses using microsatellite marker data suggested multiple separate introductions followed by population admixture in several invaded areas (Lombaert et al., 2014). The analysis also allowed the detection of 'bridgehead populations', invasive *H. axyridis* populations that served as the

source for additional invasions in other areas of the world. Lawson Handley et al. (2011) provide a useful introduction to methods used to study the ecological genetics of invasive species that could be applied to natural enemies that have been introduced—whether intentionally or unintentionally—to new geographic areas.

The molecular markers described above are useful to assay genetic variation and genetic population structure, but usually do not provide any direct indication of how the genetic variation being quantified relates to within-species variation in phenotypic traits (i.e., physiology, behaviour, life history) that determine the effectiveness of natural enemies as biological control agents (Wajnberg, 2004, 2010 provides reviews). Roush (1989, 1990), Hopper et al. (1993), Lommen et al. (2017) discuss genetic considerations in the use of entomophagous insects as biological control agents. These authors point out that most research on within-species genetic variation in insect natural enemies to date has focussed on quantifying phenotypic variation in important traits, rather than exploiting the variation to improve natural enemy performance or investigating the genetic basis of target traits. Examples of parasitoid behavioural traits that have been shown to be genetically variable include sex allocation for *Nasonia vitripennis* (Parker & Orzack, 1985; Orzack & Parker, 1990), the ability to evade encapsulation for *Leptopilina boulardi* (Carton et al., 1989), odour-conditioned ovipositor probing behaviour for *L. boulardi* (Pérez-Maluf et al., 1998), the circadian rhythm of locomotor activity for *Leptopilina heterotoma* (Fleury et al., 1995), the area searched per unit time for *Trichogramma brassicae* (Wajnberg & Colazza, 1998), patch time allocation for *Telenomus busseolae* (Wajnberg et al., 1999), the influence of mutual interference on patch exploitation behaviour in *Trissolcus basalis* (Wajnberg et al., 2004), and the temporal pattern of egg maturation in *Trichogramma brassicae* (Wajnberg et al., 2012) (Chap. 3 provides further examples). Lommen et al. (2017) provide a discussion of what traits can be assayed for variation, the the potential for selective breeding to optimise particular traits, and methods to determine the genetic architecture underlying trait variation. Chap. 3 discusses methods for studying the genetic basis of phenotypic traits in natural enemies, including the use of iso-female lines and heritability experiments.

6.5 (Mostly) Facultative Inherited Insect Symbionts

Intimate associations with beneficial infectious microorganisms have had an enormous influence on the ecology and evolution of multicellular organisms. Insects are no exception, and the past two decades have seen an explosion in the study of symbioses between insects and microbes, especially those that are passed from mothers to their offspring, often in the egg cytoplasm; this is the focus of this section. We will not cover extracellular gut symbionts here, but instead refer to some excellent reviews on that topic (Dillon & Dillon, 2004; Engel & Moran, 2013). As we will emphasise inherited symbionts that pertain most to insects as natural enemies, we will focus mainly on facultative symbioses. There are many excellent books and reviews on aspects of this topic; for readers who would like to examine this topic in more detail these include: O'Neill et al. (1997), Stouthamer et al. (1999), Moran et al. (2008), Werren et al. (2008), Engelstaedter and Hurst (2009), Oliver et al. (2010), Feldhaar (2011), Zchori-Fein and Bourtzis (2011).

Obligate symbionts

It has been estimated that at least half of all insect species host maternally inherited intracellular symbionts. These symbionts fall into two main types—obligate and facultative (Moran et al., 2008). Most obligate symbioses are nutritional and tend to be found in insects that feed on nutrient-poor diets. For example, virtually all insect lineages that feed exclusively on plant sap or animal blood host obligate nutritional symbionts that supplement their host with essential amino acids or vitamins that are missing from the

diet. Other lineages with obligate symbionts include cockroaches (their obligate *Blattabacterium* symbionts contribute to nitrogen recycling; Sabree et al., 2009), and granivorous beetles (symbionts provide additional tyrosine that is essential for cuticle thickness; Anbutsu et al., 2017). Obligate nutritional symbionts are often housed in specialised cells and organs, called bacteriocytes and bacteriomes, respectively. They commonly exhibit patterns of ancient cospeciation with their hosts, and severely reduced genomes. Due to relaxed selection and very small effective population sizes associated with host bottlenecks, they also show patterns of genome decay (McCutcheon & Moran, 2012). As a result, there have been many examples where obligate symbionts are lost and replaced with, or propped up by, another 'not-yet-as-broken' symbiont (Bennett & Moran, 2015; Sudakaran et al., 2017). Most obligate nutritional symbionts of insects are bacteria, primarily in the Gammaproteobacteria and Bacteroidetes, although there are a few examples of obligate Betaproteobacteria (e.g., *Portiera* in whiteflies, *Zinderia/Vidania/Nasuia* in the Fulgoroidea), Alphaproteobacteria (e.g., *Hodgkinia* in cicadas), and fungal symbionts (e.g., *Ophiocordyceps* in cicadas; Matsuura et al., 2018).

There are hardly any examples of obligate inherited symbionts of insect parasitoids or predators, and we are not aware of any that involve nutrition. Philanthine beewolves harbour obligate symbiotic *Streptomyces* bacteria in glands in their antennae (Kaltenpoth et al., 2005). The bacteria protect wasp larvae against pathogenic soil fungi using a complex cocktail of antibiotics. The association between beewolves and *Streptomyces* symbionts is at least 60 million years old but has not followed a pattern of strict cospeciation, as some wasp species have exchanged symbionts (Kaltenpoth et al., 2014).

As far as we are aware, no obligate insect symbionts have been directly implicated in protection against parasitoids or predators. This is perhaps not so surprising, as their tiny genomes mostly retain genes involved in nutrient production, although one interesting exception is *Profftella armatura*, an obligate Betaproteobacterial symbiont of the Asian citrus psyllid, *Diaphorina citri*. Although it has a tiny genome, about one-sixth of its genome is composed of genes involved in the biosynthesis of polyketide toxins showing potent toxicity against human HeLa and rat neuroblastoma cells (Nakabachi et al., 2013). It is not yet known whether these toxins are deployed in the wild and against what enemy.

Facultative symbionts

Facultative symbionts, i.e., symbionts that are not essential for the reproduction and survival of their insect host, are more common than obligate symbionts (O'Neill et al., 1997; Stouthamer et al., 1999; Moran et al., 2008; Werren et al., 2008; Engelstadter & Hurst, 2009) and are the focus of the rest of the chapter. Unlike obligate symbionts, which appear to be restricted to certain lineages depending on host diet or metabolism, facultative inherited symbionts occur in every order of insect. Although they are primarily vertically transmitted over ecological timescales, their long-term evolutionary patterns differ from obligate inherited symbionts, as they do not exhibit ancient cospeciation. Instead there is little concordance between host and symbiont phylogenies, so that unrelated hosts often harbour closely related symbionts, and related hosts often differ in their symbiont status. As we will discuss below, one of the major unresolved questions in the field is how facultative inherited symbionts infect new hosts, and what determines their suitability and successful establishment in a new host. Also, while it is clear that there is much horizontal transmission over evolutionary timescales, it is not at all clear how important or common horizontal transmission is over ecological timescales; this is likely to be very much dependent on the specific symbiont.

Vertical transmission has very important consequences for the ecology and evolution of symbiosis. The fitness of a vertically transmitted symbiont is directly tied to the fitness of its host, and so this bears directly on the strategies that symbionts have evolved, from increasing the fitness of their hosts under certain conditions, to manipulating host reproduction in order to increase the frequency of infected females. So a

symbiont's effect on its host can give us important clues as to how it is transmitted, and vice versa.

The prevalence and persistence of a symbiont that is primarily maternally transmitted will largely depend on two main factors: its effect on host fitness, and how efficiently it is transmitted. Facultative inherited symbionts vary widely in prevalence in their hosts, and there can often be major differences in prevalence between different populations, for example between native versus invasive ranges (e.g., Nguyen et al., 2016). Fitness effects can be incredibly hard to measure, especially in the field. Fitness differences can be subtle, and in the laboratory, might only be uncovered in population cages or under stressful conditions (e.g., Oliver et al., 2008).

Vertical transmission efficiency is often high in the laboratory, but can be much lower in the wild, where all sorts of abiotic and biotic factors, such as varying temperature or the presence of other symbionts could interfere with transmission (e.g., Turelli & Hoffmann, 1995; Rock et al., 2018). In general, mechanisms regulating transmission efficiency and symbiont titre within a host are not well understood. Facultative symbionts have evolved a number of different strategies to ensure efficient germline transmission (Russell et al., 2009). For example, *Spiroplasma* that infect *Drosophila* target yolk protein to enter oocytes (Herren et al., 2013), and *Wolbachia* have an affinity for stem cells (Frydman et al., 2006). Symbiont titres can vary widely within individuals (Unckless et al., 2009), but the consequences of titre variation on either transmission or fitness are not well understood (but see Martinez et al., 2014).

Symbiont dynamics will be completely altered when there is horizontal transmission. Indeed, many infectious microorganisms have mixed modes of transmission (Ebert, 2013), and we might expect that those with mixed transmission have less efficient vertical transmission. Once the link between host and symbiont fitness is weakened, strong fitness benefits are no longer required to explain persistence, and we begin to see and expect stronger costs of infection, and possibly pathogenicity. So it is very important to consider the extent to which horizontal transmission occurs on ecological timescales, and how important is horizontal transmission in explaining symbiont persistence. Non-maternal routes of transmission that have been experimentally confirmed in facultative inherited symbionts include sexual transmission (Moran & Dunbar, 2006), transmission via plants (Caspi-Fluger et al., 2012), via blood of shared hosts (Hirunkanokpun et al., 2011), and from parasitoid to parasitoid in a shared host (Huigens et al., 2000). Parasitoids and parasitic mites have also been shown to transfer symbionts between hosts (Jaenike et al., 2007; Gehrer & Vorburger, 2012).

6.5.1 Reproductive Parasitism

Facultative inherited symbionts are very well known for their ability to manipulate reproduction to either increase the frequency of infected females (i.e., the transmitting sex) or to decrease the frequency of uninfected females. There are four common types of reproductive manipulation: cytoplasmic incompatibility, and three different types of sex-ratio distortion—male-killing, feminisation, and parthenogenesis-induction. For more detailed information about reproductive manipulation, there are a number of excellent reviews, including Werren and O'Neill (1997), Stouthamer et al. (1999), Werren et al. (2008), Engelstaedter and Hurst (2009), Drew et al. (2019). It should be pointed out that maternally inherited symbionts are predicted to bias their transmission in many interesting ways that remain to be discovered (Hurst, 1993; Werren & O'Neill, 1997). For example, a recent study demonstrated for the first time that *Wolbachia* can interfere with the proper inheritance of sex chromosomes in butterflies (Kageyama et al., 2017). Reproductive manipulators can also benefit their hosts in ways that are independent of reproductive manipulation (Drew et al., 2019). For example, male-killing *Wolbachia* and *Spiroplasma* have been shown to protect their hosts against natural enemies in the laboratory (Unckless & Jaenike, 2012; Xie et al., 2014).

A long-term challenge is to understand the relative importance of reproductive manipulation versus other fitness effects in nature.

Cytoplasmic incompatibility

Cytoplasmic incompatibility, or CI, is the reproductive manipulation that is the most widespread and that has received by far the most attention. Cytoplasmic incompatibility results from mating incompatibilities between infected males and uninfected females, fitting a toxin-antitoxin model, with the inherited microbe producing a toxic factor that damages or modifies male sperm, and an antidote in females (Weisser & Völkl, 1997; Beckmann et al., 2019). Uninfected females are therefore at a severe disadvantage. Without the antidote, their offspring die early in development and, as a result, uninfected females can be quickly replaced by infected ones, and suffer a growing disadvantage every generation as they encounter fewer and fewer compatible and uninfected males to mate with (Turelli, 1994). Indeed, a number of studies have documented the very rapid spread and replacement of insect populations with a CI-microbe infected strain (Turelli & Hoffmann, 1991; Turelli et al., 2018). A variant of CI has also been documented in some haplodiploid insects, such as the parasitoid wasp *Nasonia vitripennis*, whereby uninfected females that mate with infected males produce a very heavily male-biased offspring sex ratio (Ryan & Saul, 1968; Breeuwer & Werren, 1990). This is because the paternal 'poisoned' chromosomes die, but the eggs develop as haploid males from chromosomes they inherited from their mother.

Despite affecting a wide range of arthropods, only a handful of bacteria are known to cause cytoplasmic incompatibility (Yen & Barr, 1971, 1973; Hunter et al., 2003; Takano et al., 2017). By far the most common is *Wolbachia*, and it has been shown to be able to do this across an incredibly wide range of species and orders, suggesting a general mode of action of poisoning sperm and rescuing eggs. A huge, and recent, development in the field has been the long-awaited discovery of the toxin-antitoxin factors that cause CI, called cifA and cifB (or cidA and cidB) (Lepage et al., 2017; Shropshire et al., 2018; Beckmann et al., 2017). Cif genes are encoded in *Wolbachia* prophages that are highly mobile across *Wolbachia* genomes, and are also frequently degraded or lost (Lindsey et al., 2018), explaining why closely related *Wolbachia* differ in the ability to induce CI. A next major step will be to determine how these factors interact with the host. This will help address the as yet unresolved mystery of why some hosts appear to be more resistant to CI than others, and why some recently invaded strains do not appear to cause CI, despite having apparently functional cif genes (Turelli et al., 2018).

More recently, the symbiont *Cardinium* was shown to also cause cytoplasmic incompatibility in four arthropod orders, Hymenoptera (Hunter et al., 2003), Acari (Gotoh et al., 2007), Hemiptera (Nakamura et al., 2012) and Thysanoptera (Nguyen et al., 2017). The mechanism is completely different, as its genome does not contain cif genes (Penz et al., 2012). Finally, an unnamed alphaproteobacterium has been implicated in CI in a beetle pest of coconut, *Brontispa longissima* (Takano et al., 2017), but only a single paper about this has so far been published.

CI microbes are relevant for researchers studying insect natural enemies for a number of reasons. They have the potential to quickly transform a species, and may explain strange patterns of incompatibility and egg hatch failure when working with insects that differ in infection status. They can also contribute to interesting mate discrimination patterns that may lead to reproductive isolation. This has perhaps best been demonstrated in a species pair of woodland flies, *Drosophila recens* and *D. subquinaria* (Jaenike et al., 2006), with the former infected with a strain of *Wolbachia* that causes strong CI. The two species have a broad range of overlap, and when female *D. subquinaria* mate with male *D. recens*, few viable offspring are produced. As a result, where they overlap, *D. subquinaria* shows strong mating avoidance with *D. recens*, and a striking by-product of this discrimination is that allopatric mating is also avoided.

Even before its causal agent was known, cytoplasmic incompatibility was used for pest

control: Hannes Laven (1967) released incompatible *Culex pipiens* male mosquitoes in Rangoon (Yangon), where they mated with uninfected females in a manner analogous to sterile insect male release. Incompatible males can also be generated in the laboratory by embryonic injection (Zabalou et al., 2004; O'Connor et al., 2012; Cattel et al., 2018). In another control approach, *Aedes aegypti* mosquitoes have been transfected with a strain of *Wolbachia* from *Drosophila* that causes CI and also suppresses RNA viruses, including dengue (Hoffmann et al., 2011; Walker et al., 2011). Female mosquitoes were released in the wild, where they quickly spread through the population as a result of CI, bringing virus suppression ability along for the ride.

Male-killing

Male-killing by maternally transmitted bacteria or viruses has evolved independently at least six times, including *Wolbachia*, *Rickettsia*, *Spiroplasma*, *Arsenophonus*, and *Flavobacterium*, with killing typically occurring early in embryonic development (Hurst & Jiggins, 2000). Most male-killers are found at low prevalence, often due to incomplete vertical transmission and reduced fitness of infected females. But there are exceptions, such as the nymphalid butterfly *Acraea encedon*, with over 95% of females infected with a male-killing *Wolbachia* in the wild (Jiggins et al., 2002). A number of studies have looked for fitness benefits of male-killing infections, for example, testing whether infected females are larger, possibly as a result of reduced competition over resources, but fitness benefits have proven elusive (but see Koop et al., 2009). Male-killing is especially widespread in ladybird beetles. Ladybirds lay their eggs in clutches and sibling cannibalism is common. Newly hatched infected females thus gain a direct fitness benefit from feeding on their dead brothers (Osawa, 1992; Nakamura et al., 2006).

Alternatively, male-killers may offer another fitness benefit that is not directly related to male-killing, and this may be important in explaining their persistence in the wild.

A few studies have documented rapid evolution by insects to suppress male-killing, such as in the predatory green lacewing, *Mallada desjardini* (Hayashi et al., 2018). If suppression is common, then male-killers may be more widespread than appreciated, but hidden. As an example, when a strain of *Wolbachia* that causes CI in *Drosophila recens* was introgressed into *D. subquinaria*, it caused male-killling in some but not all lines (Jaenike, 1997), demonstrating that there is segregating genetic variation in *D. subquinaria* to suppress male-killing (and that this trait is fixed in *D. recens*). Researchers demonstrated rapid evolution of host suppression of male-killing by *Wolbachia* in the butterfly *Hypolimnas bolimna* (Hornett et al., 2006). Suppression, however, did not result in elimination of the *Wolbachia*, because it also causes cytoplasmic incompatibility (Hornett et al., 2008), further demonstrating a link between CI and male-killing in *Wolbachia*. Little else is known about the mechanism of male-killing in general, and it is striking that symbionts have evolved the ability to kill males in arthropod lineages with incredibly diverse modes of sex determination. A recent breakthrough identified the factor that causes male-killing in the *Spiroplasma* symbiont of *Drosophila melanogaster,* a toxin called spaid (Harumoto & Lemaitre, 2018).

Sex ratio distortion via *parthenogenesis-induction* and *feminisation*

A number of symbionts cause an increase in the prevalence of females by transforming males into females. This has been most studied and is widespread in isopods and amphipods (Bouchon et al., 1998; Terry et al., 2004), where microsporidia and *Wolbachia* feminise males by suppressing the development of the male-determining androgenic gland. Feminising *Wolbachia* have also been documented in butterflies and moths (Hiroki et al., 2002; Kageyama et al., 2002) and leafhoppers (Negri et al., 2006). *Cardinium* feminises the false spider mite *Brevipalpus phoenicis*, resulting in the only documented case of haploid females in animals (Weeks et al., 2001).

Three symbionts have independently evolved the ability to induce parthenogenesis in their hosts—*Wolbachia*, *Cardinium*, and *Rickettsia*. Parthenogenesis-induction (or PI) is especially common in haplodiploids (Ma & Schwander, 2017), having been conclusively demonstrated in Hymenoptera (Stouthamer et al., 1990), Acari (Weeks & Breeuwer, 2001), and Thysanoptera (Arakaki et al., 2001). The tight link between PI and haplodiploidy stems from two major reasons. First, some types of haplodiploid sex determination are particularly vulnerable to PI microbes, requiring the conversion of unfertilised eggs from haploid to diploid. This has been shown to result via gamete duplication, where meiosis proceeds normally but chromosomes fail to separate following an aborted mitosis (e.g., Stouthamer & Kazmer, 1994), or by elimination of meiosis altogether (Adachi-Hagimori et al., 2008). Second, PI may be easier to demonstrate in haplodiploids because antibiotic treatment (often, but not always) results in viable haploid males. A number of diploid parthenogenetic insects are fixed for inherited symbiont infection but antibiotic treatment results in sterility (e.g., Yusuf & Turner, 2004; Pike & Kingcombe, 2009), and so it is more challenging to confirm that the symbiont is the causal agent. In the whitefly parasitoid *Encarsia hispida*, which is infected with *Cardinium*, antibiotic treatment results in diploid males, demonstrating that the lines between PI and feminisation can sometimes be blurred (Giorgini et al., 2009).

With few exceptions, PI infections are rapidly fixed because the short-term benefit of asexual reproduction is so great. In addition, as PI infections in haplodiploids spread, they are predicted to select for refractoriness to mating (Stouthamer, 1997). This is because haplodiploid females that do not mate will produce only sons, which will be at a reproductive advantage as PI initially spreads through the population. Curing insects of their PI infection will cause major problems—often resulting in refractory females or sterile males that have acquired deleterious mutations in genes that are important in sexual reproduction and/or male function (Gottlieb & Zchori-Fein, 2001; Jeong & Stouthamer, 2005; Pannebakker et al., 2005). Thus there is reduced gene flow between PI-infected lineages and their sexual relatives. Interestingly, polymorphic lineages containing parthenogenetic (and symbiont-infected) and sexual uninfected members are very common, and these often show differences in their geographic distribution (Ma & Schwander, 2017).

6.5.2 Conditional Mutualism, with a Focus on Phenotypes Affecting Insect Natural Enemies

Many facultative inherited symbionts increase the fitness of their hosts under certain conditions, for example increasing a herbivore's plant host range (Tsuchida et al., 2004; Wagner et al., 2015) or helping a host tolerate various abiotic stresses, such as high temperature (Montllor et al., 2002). In the following section, we will focus on phenotypes affecting insect natural enemies, either with respect to protecting hosts against parasitoids or predators, or with respect to symbionts that affect a parasitoid or predator's host use.

Defensive symbionts

A number of inherited symbionts have been shown to protect insects against a wide range of natural enemies, from microbial pathogens, such as fungi (Scarborough et al., 2005) and viruses (Hedges et al., 2008; Teixeira et al., 2008), to parasitic nematodes (Jaenike et al., 2010), parasitic wasps (Oliver et al., 2003) and predators (Kellner, 2001). Protection can occur in diverse ways (Haine, 2008; Florez et al., 2015; Vorburger & Perlman, 2018). First, symbionts may produce toxins that directly harm the natural enemy. This is a very common type of protection, and one that is likely to promote specificity and coevolutionary interactions. Second, symbionts may trigger a host immune response that targets the natural enemy, such as in recently established strains of *Wolbachia* in mosquitoes (Moreira et al., 2009). Finally, symbionts may negatively affect a natural enemy by depleting it of a limiting essential nutrient, such as

Wolbachia that compete with viruses over cholesterol (Caragata et al., 2013). There are also a few examples of symbionts that prevent infection or attack. For example, infection with a strain of *Rickettsiella* changes the colour of their pea aphid hosts (Tsuchida et al., 2010), resulting in less predation by ladybird beetles (Polin et al., 2015). Finally, plants fed on by facultative symbiont-infected aphids release fewer volatiles, attracting fewer parasitoids than plants fed on by symbiont-free aphids (Frago et al., 2017).

We are only aware of one example of an inherited insect symbiont that provides protection against predators. This was the first documented insect defensive symbiosis (Kellner, 2001). A number of rove beetles in the subfamily Paederinae are well known for being highly toxic; handling them can cause terrible blistering, due to toxic polyketides called pederins that are produced by endosymbiotic *Pseudomonas* bacteria (Kellner, 2002a; Piel, 2002). *Pseudomonas* symbionts acquired pederin synthesis genes via horizontal transfer; symbionts of sponges (Piel et al., 2004) and psyllids (Nakabachi et al., 2013) have also acquired related poisons. Pederins protect rove beetle larvae from spiders (Kellner & Dettner, 1996). Beetles are often aposematically coloured, and some individuals in the wild appear to be symbiont free (Kellner & Dettner, 1995), presumably benefiting from the protection conferred by their infected conspecifics. Mothers secrete bacteria over the surface of eggs. Uninfected larvae can also acquire symbionts by feeding on infected eggs, and although this does not appear to protect them, they can then pass symbionts on to their offspring (Kellner, 1999). Horizontal transmission has been shown to occur both intra- and inter-specifically (Kellner, 2002b).

Symbiont-mediated protection against parasitoids has been studied in much greater detail, particularly in aphids (Oliver et al., 2014). By far the most work has been done on the aphid facultative symbiont *Hamiltonella defensa*. Protection is due to diverse toxins that are encoded by phages (Oliver et al., 2009; Brandt et al., 2017), and related phages and toxins have been found in *Arsenophonus* symbionts (Duron, 2014), including ones that have been correlated with parasitoid presence (Hansen et al., 2007), suggesting a role in protection for this symbiont as well. Protection by *Hamiltonella* confers a powerful benefit, and in both experimental population cages and field experiments (Oliver et al., 2008; Rothacher et al., 2016), has been shown to rapidly alter population dynamics, decreasing susceptible wasp abundance. However, in the absence of wasp infection, the presence of *Hamiltonella* (or *Regiella*, a related aphid defensive symbiont) imposes a strong fitness to the aphid host (Oliver et al., 2008; Vorburger & Gouskov, 2011). Researchers have also demonstrated a high level of specificity in protection and in wasp counter-resistance that has shaped coevolutionary interactions between host, symbiont and wasp. This results in dynamic spatial and temporal variation in symbiont infection status and strain diversity, and in wasp community composition (e.g., Vorburger et al., 2009; Oliver et al., 2012; Smith et al., 2015; Mclean & Godfray, 2017).

Spiroplasma symbionts, including male-killing strains, have also been shown to protect *Drosophila* flies against parasitic wasps in the laboratory (Xie et al., 2010, 2014), although it is not yet known whether this is important in nature. Two mechanisms have been implicated in protection: resource competition over host lipids (Paredes et al., 2016) and toxins. *Spiroplasma* produce a wide range of toxins called ribosome-inactivating proteins (RIPs) that are homologous to well-known plant and bacterial poisons such as ricin and Shiga toxin, respectively (Hamilton et al., 2016). These poisons target a highly conserved adenine in eukaryotic 28S ribosomal RNA, and, in *Spiroplasma*-infected hosts, wasp ribosomes shown a characteristic signature of RIP attack (Ballinger & Perlman, 2017). It is not yet known how RIPs enter wasp cells, or why host ribosomes are relatively unaffected. A recent study documented wasp species that are resistant to *Spiroplasma* protection, suggesting that there may be coevolutionary interactions between wasps and *Spiroplasma* (Mateos et al., 2016).

We are not aware of any biological control programmes that have been shown to be

impacted by defensive symbionts, but given how strong the benefit to the pest can be, this should certainly be considered, especially where biological control failure and/or rapid evolution of resistance have been reported. For example, a recent study documented rapid decline in the success of an introduced *Microctonus* wasp against an exotic weevil in New Zealand (Tomasetto et al., 2017). It is not known if this biocontrol failure is due to a cryptic endosymbiont, although an earlier survey found diverse facultative symbionts in pest weevils (White et al., 2015). In an experimental test of the effect of defensive symbionts on the efficacy of biological control, control by *Lysiphlebus* wasps against black bean aphids collapsed in the span of months, because of strong selection for *Hamiltonella*-infected aphid clones (Kach et al., 2018).

Symbionts of natural enemies that affect host use or host range

There are few examples of inherited symbionts of natural enemies that affect host usage or host range, and this would be interesting to look into in more detail. For example, we are not aware of any cases of insect 'offensive' bacterial symbionts that aid in attacking a host, similar to *Photorhabdus* and *Xenorhabdus* symbionts of entomopathogenic nematodes that kill and then protect prey items from other competitors. Perhaps the best example of a symbiont affecting host behaviour is a strain of *Cardinium* that infects the whitefly parasitoid *Encarsia tabacivora* (formerly *Encarsia pergandiella*). Most *Encarsia* have an unusual host-use behaviour, called autoparasitism, whereby (diploid) female eggs are laid in whitefly or scale insect hosts, and (haploid) male eggs are laid inside developing wasps (i.e., males are hyperparasitoids). Interestingly, infection with a parthenogenesis-inducing *Cardinium* affects female oviposition behaviour. Parthenogenetic females oviposit equally in whitefly and wasp hosts, but upon antibiotic treatment, strongly prefer laying eggs in wasps (Zchori-Fein et al., 2001; Kenyon & Hunter, 2007).

An especially promising place to look for host manipulation is in inherited viruses of parasitoids (e.g., Renault et al., 2005), such as the well-known obligate symbiotic polydnaviruses of braconids and ichneumonids (Strand & Burke, 2013). This is an exciting area of research, with genomics helping to discover diverse and interesting infections. For example, an intriguing RNA virus was recently discovered in *Dinocampus coccinellae*, a braconid wasp that parasitises ladybird beetles (Dheilly et al., 2015). The virus is injected into hosts along with wasp eggs, replicates in beetle nervous tissue, and is associated with beetle paralysis. In another interesting example of manipulation, Varaldi and colleagues discovered an inherited DNA virus of *Leptopilina* wasps that modifies wasp behaviour, such that infected females are more likely to superparasitise hosts (Varaldi et al., 2003), increasing the likelihood of horizontal transmission.

6.5.3 Taxonomic Distribution of Facultative Inherited Symbionts

In the following section, we will briefly introduce the main lineages of facultative inherited symbionts of insects. Most facultative inherited symbionts are bacteria, with five extraordinarily widespread lineages, *Wolbachia*, *Cardinium*, *Rickettsia*, *Arsenophonus*, and *Spiroplasma* (Duron et al., 2008a, b), and a number that are less studied and/or less widespread. Although bacterial symbionts are probably more common than other types of microbial symbionts, there has also been a strong bias towards their discovery, due to 'universal' primers that target bacterial 16S rRNA, and because of the availability of antibiotics to remove them. However, one should be mindful that there may be bacterial symbionts that have been missed because of unusual 16S rRNA sequences (Brown et al., 2015a, b), or that are difficult to cure with antibiotics. Inherited viruses, or microbial eukaryotes, such as yeasts or fungi (Gibson &

Hunter, 2010), have been much less studied, and this is an emerging frontier.

Wolbachia

The alphaproteobacterium *Wolbachia* is by far the most important and most studied facultative symbiont, for two good reasons. First, the sheer number of terrestrial arthropods that it infects is astounding. It has been estimated to infect ~40–50% of all terrestrial arthropods, making it perhaps the most abundant microbe on the planet (Zug & Hammerstein, 2012; Weinert et al., 2015). In addition to terrestrial arthropods, about 40% of filarial nematode species harbour *Wolbachia* (Bandi et al., 1998; Ferri et al., 2011), and it was recently discovered in the plant-parasitic nematodes *Radopholus similis* (Haegeman et al., 2009) and *Pratylenchus penetrans* (Brown et al., 2016).

Second, no other symbiont comes close in terms of the range of effects that *Wolbachia* strains exert on their hosts. *Wolbachia* is best known as a reproductive manipulator, with various strains causing cytoplasmic incompatibility, male-killing, parthenogenesis-induction and feminisation across a wide range of arthropod orders. Kageyama et al. (2017) recently showed that *Wolbachia* interferes with sex chromosome inheritance in the butterfly *Eurema mandarina*, the first demonstration of a symbiont that causes meiotic drive. This should serve to highlight the fact that not only are there new types of reproductive manipulation to discover, but that we still do not understand how the vast majority of *Wolbachia* infections persist in their hosts. Many strains either do not show reproductive manipulation or are weak reproductive manipulators. For example, the strain that infects *D. melanogaster* causes weak cytoplasmic incompatibility that is not strong enough on its own to explain its persistence, and this has motivated much research into identifying other host effects, for example, increasing fecundity under periods of iron stress (Brownlie et al., 2009). Indeed, many reproductive manipulators can increase host fitness in various, often conditional, contexts (Drew et al., 2019), although a long-term challenge will be to determine whether these fitness benefits occur in the wild (Zug & Hammerstein, 2015).

A major development since the second edition of this book has been the discovery that some strains of *Wolbachia* protect their hosts against positive-sense RNA viruses (Hedges et al., 2008; Teixeira et al., 2008; Moreira et al., 2009). This was first discovered in *Drosophila* and has spurred a global initiative to control dengue, Chikungunya and Zika virus, by transfecting *Aedes aegypti* mosquitoes with *Wolbachia* strains that cause both cytoplasmic incompatibility and virus suppression, and releasing them into the wild (Hoffmann et al., 2011; Walker et al., 2011; O'Neill, 2018). Since the initial releases in northern Queensland, Australia, there have not been any reported cases of dengue there (O'Neill, 2018), and a randomised control trial to determine whether *Wolbachia* releases have reduced dengue is underway in Indonesia (Anders et al., 2018).

How *Wolbachia* suppresses positive-sense RNA viruses is not currently known, and is an active area of research (Caragata et al., 2013; Bhattacharya et al., 2017; Geoghegan et al., 2017; Lindsey et al., 2018; Schultz et al., 2018; Thomas et al., 2018). While protection has been shown in the laboratory and in transfected mosquitoes in the wild, as far as we are aware, there is not any evidence demonstrating protection in a natural system in the wild (Shi et al., 2018). Protection in insects other than *Drosophila* or mosquitoes is largely unstudied but *Wolbachia* has been shown to cause increased susceptibility to nucleopolydrovirus in the African armyworm *Spodoptera exempta* (Graham et al., 2012).

Not all *Wolbachia* are facultative. A divergent *Wolbachia* has evolved to become the obligate nutritional symbiont of bedbugs, where it is housed in bacteriomes and provides essential B vitamins (Hosokawa et al., 2010). *Wolbachia* is required for oogenesis in the braconid parasitoid wasp, *Asobara tabida*, as its removal causes sterility (Dedeine et al., 2001). This strain has been implicated in interfering with the progression of programmed cell death that is required for the proper development of eggs (Pannebakker et al., 2007). *Wolbachia* is also essential in

filarial nematodes (Bandi et al., 1999; Hoerauf et al., 1999), but the reason for this is not well understood, particularly because many filarial nematode species do not harbour *Wolbachia* (Ferri et al., 2011), nor have they acquired other symbionts or novel metabolic genes (Desjardins et al., 2013). Instead, *Wolbachia* appears to be required for proper germline development (Foray et al., 2018), in a manner that is reminiscent of what is seen in *Asobara*. Thus, *Wolbachia* appears to have exploited its ability to manipulate germline to become essential in *Asobara* wasps and filarial nematodes.

A major unresolved pair of questions in the study of *Wolbachia* concern how and how much it colonises new species. From phylogenetic analyses, it is clear that there is a great deal of horizontal transmission, with closely related strains found in unrelated hosts (Werren et al., 2008). Species that share a food resource, parasitoids and their hosts, and predators and their prey, have all been shown to harbour related *Wolbachia* (e.g., Vavre et al., 1999; Stahlhut et al., 2010), suggesting ecological routes of transmission. Despite the many examples of distant host switches, there is also a great deal of phylogenetic signal in *Wolbachia* infection, with related strains often clustering in closely related hosts (Russell et al., 2009). This may be due to cospeciation, introgression, or horizontal transmission, and without extensive sequencing it may be difficult to distinguish between these alternatives (Turelli et al., 2018). Also, new infections are more likely to establish if they occur in closely related hosts.

Finally, although *Wolbachia* has such a wide host range with extensive horizontal transmission over evolutionary timescales, experimental examples of ecologically realistic horizontal transmission are exceedingly rare. In the best example of ecological transmission of *Wolbachia*, Huigens et al., (2000, 2004) showed that uninfected *Trichogramma* parasitic wasps can acquire parthenogenesis-inducing *Wolbachia* from infected wasps developing in the same moth egg. It has been reported that *Bemisia tabaci* whiteflies can acquire *Wolbachia* from both parasitoids and plants (Ahmed et al., 2015; Li et al., 2017), but the donor and recipient in both studies was the same species with the same mitochondrial haplotype, so it is difficult to rule out laboratory artefacts. It is important to note that establishing novel *Wolbachia* infections typically requires laborious embryonic microinjection, with very low success rates.

Cardinium

Cardinium was the last of the five major facultative symbionts to be discovered (Weeks et al., 2001; Zchori-Fein et al., 2001, 2004). It is also the only one in the phylum Bacteroidetes, which is a source of many obligate symbionts in insects (e.g., Bandi et al., 1994; Moran et al., 2005; Chong & Moran, 2018; Engl et al., 2018). The closest known relative of *Cardinium* is a facultative symbiont of amoeba, *Amoebophilus* (Horn et al., 2001). *Cardinium* is estimated to infect ~5–10% of terrestrial arthropods (Weeks et al., 2003; Zchori-Fein & Perlman, 2004; Duron et al., 2008a; Weinert et al., 2015); infections are especially common in some arthropod lineages, such as chalcidoid wasps (Zchori-Fein et al., 2001), biting midges (Nakamura et al., 2009), mites (Gotoh et al., 2007), and spiders (Duron et al., 2008b). It has also been reported in the plant-parasitic nematodes *Pratylenchus penetrans*, *Globodera pallida*, and *Heterodera glycines* (Shepherd et al., 1973; Noel & Atibalentja, 2006; Brown et al., 2018). *Cardinium* strains exhibit a distinct morphology that is discernible with electron microscopy (Zchori-Fein et al., 2001), with microfilament-like structures attached to membrane. The function of these structures is not known in *Cardinium*, but in *Amoebophilus* they were recently shown to form part of a secretion system that injects effectors into host cells (Bock et al., 2017).

How *Cardinium* affects its hosts has only been demonstrated in a handful of cases, but it has received the most attention for causing diverse reproductive manipulations, including parthenogenesis and feminisation in parasitic wasps (Zchori-Fein et al., 2004; Giorgini et al., 2009) and mites (Weeks et al., 2001). *Cardinium* also causes cytoplasmic incompatibility in parasitic wasps (Hunter et al., 2003; Gebiola et al., 2016),

mites (Gotoh et al., 2007), planthoppers (Nakamura et al., 2012) and thrips (Nguyen et al., 2017), and this represents an interesting counterpoint and comparison to cytoplasmic incompatibility induced by *Wolbachia*. The genome and transcriptome of a CI-inducing *Cardinium* strain were recently sequenced, and it does not appear to share genes with *Wolbachia*, suggesting that the mechanisms of cytoplasmic incompatibility are completely different (Penz et al., 2012; Mann et al., 2017).

Rickettsia

The alphaproteobacterial symbiont *Rickettsia* is another extremely widespread facultative symbiont, estimated to infect ~ 5–20% of terrestrial arthropods from a wide range of orders (Perlman et al., 2006; Duron et al., 2008a, b; Weinert et al., 2009, 2015); it also occurs in glossiphoniid leeches (Kikuchi et al., 2002). Although the best-known *Rickettsia* cause diseases in humans and are vectored by blood-feeding arthropods, such as lice, fleas, and ticks, these represent just a few branches on the *Rickettsia* phylogenetic tree. Most *Rickettsia*, however, are primarily maternally transmitted symbionts of insects that do not blood-feed, with host effects that are largely unknown. *Rickettsia* are male-killers in coccinellid and buprestid beetles (Werren et al., 1994; Lawson et al., 2001), and cause parthenogenesis in eulophid parasitic wasps (Hagimori et al., 2006; Giorgini et al., 2010). *Rickettsia* was also recently implicated in feminisation in the linyphiid spider, *Mermessus fradeorum* (Curry et al., 2015).

An inherited *Rickettsia* in the sweet potato whitefly *Bemisia tabaci* was recently found to rapidly spread across the southwestern United States, with the prevalence of *Rickettsia*-infected whiteflies increasing from 1 to 97% in just six years (Himler et al., 2011). *Rickettsia* resulted in higher whitefly fecundity and female-biased sex ratios, but what caused the fitness increase and sex ratio distortion is not known. The fitness benefit appeared to be conditional, and was affected by host genotype (Cass et al., 2016; Hunter et al., 2017) and environment (Cass et al., 2015). This strain was also found to protect whiteflies against entomopathogenic bacteria (Hendry et al., 2014). Variable effects of *Rickettsia* have also been reported in pea aphids. Infected aphids weigh less and produce fewer offspring (Sakurai et al., 2005) but are protected against pathogenic fungi (Lukasik et al., 2013).

Since the best-known *Rickettsia* can be horizontally transmitted from blood-feeding arthropods to vertebrates, it should perhaps not be surprising that ecological horizontal transmission has been demonstrated in a number of *Rickettsia*. Uninfected cat fleas can acquire *R. felis* by co-feeding on the same vertebrate host as infected conspecifics (Brown et al., 2015a, b; Hirunkanokpun et al., 2011), and the authors suggest that horizontal transmission is required for persistence of the infection as vertical transmission is quite inefficient (Wedinkamp and Foil, 2002). Co-feeding with uninfected rat fleas resulted in interspecific transmission. *Bemisia tabaci* whiteflies have been shown to acquire *Rickettsia* through phloem (Caspi-Fluger et al., 2012), although this mode of transmission was ruled out in the case of rapid spread of *Rickettsia* (Himler et al., 2011). Finally, in the leafhopper *Nephotettix cincticeps*, *Rickettsia* shows both maternal and paternal transmission, and not only does it reside in sperm cytoplasm but it also invades nuclei (Watanabe et al., 2014).

Arsenophonus

Arsenophonus, in the Enterobacteriaceae (Gammaproteobacteria), is also widespread and relatively understudied, infecting ~5% of insects across a broad range of orders (Duron et al., 2008a, b; Novakova et al., 2009). A few *Arsenophonus* lineages have evolved into obligate nutritional symbionts of blood-feeding insects, such as human lice and their relatives (where they have also been called *Riesia*), and louse flies and bat flies. Only a handful of facultative inherited *Arsenophonus* have been implicated in any host phenotypes. In the invasive psyllid *Glycaspis brimblecombei*, the presence of an *Arsenophonus* symbiont is highly correlated with the prevalence of parasitism by encyrtid wasps (Hansen et al., 2007). This symbiont harbours a phage that is related to the

APSE phage found in *Hamiltonella* defensive symbionts of aphids and that provide protection against parasitic wasps. Many *Arsenophonus* strains harbour APSE phages, suggesting that defence by *Arsenophonus* may be common (Duron, 2014).

A number of *Arsenophonus* have interesting associations with plants. In the cowpea aphid *Aphis craccivora*, *Arsenophonus* increases plant host range, permitting development on locust plants (Wagner et al., 2015). Two unrelated strains of *Arsenophonus* cause diseases in strawberries (called marginal chlorosis) and beets (called SBR, or syndrome basses richesses) and are vectored by planthoppers (Danet et al., 2003; Semetey et al., 2007; Salar et al., 2010; Bressan et al., 2012). These plant-pathogenic *Arsenophonus* are transmitted both vertically and horizontally (Bressan et al., 2009).

The only *Arsenophonus* known to be a reproductive manipulator is worth special mention because it is so unusual. *Arsenophonus nasoniae*, also known as 'son-killer', kills male *Nasonia vitripennis* and other chalcidoid parasitoids that attack fly pupae (Werren et al., 1986; Duron et al., 2010). But *A. nasoniae* is neither intracellular nor transmitted in eggs. Instead, adult female wasps inject the bacteria into fly pupae when they oviposit. Wasp larvae then ingest the bacteria, which kill ~80% of developing male larvae (Huger et al., 1985; Skinner, 1985). Bacteria can also therefore be ingested by and transmitted to uninfected broods, as well as different wasp species (Duron et al., 2010) developing in the same fly pupa. Indeed, it was recently shown that son-killer cannot persist in population cages without ample horizontal transfer via superparasitism (Parratt et al., 2016). *Arsenophonus nasoniae* is also unusual in that it is relatively easy to culture (Gherna et al., 1991).

Spiroplasma

Spiroplasma is a member of the *Mollicutes*, a class of gram-positive bacteria that consists entirely of host-associated microbes, including *Mycoplasma* and *Phytoplasma*, common animal and plant parasites, respectively (Anbutsu & Fukatsu, 2011; Ballinger & Perlman, 2019). *Mollicutes* lack a cell wall and are therefore missing the typical bacterial elicitors of animal and plant immune systems, which helps explain why they are such successful infectious agents. It is difficult to estimate the prevalence of inherited *Spiroplasma* for a few reasons, although it has been estimated at ~5% (Duron et al., 2008a, b). First, because *Spiroplasma* is old, diverse, and paraphyletic, diagnostic primers may be more appropriate for specific species groups. Second, many *Spiroplasma* reside in the gut, as commensals or pathogens. Well-studied pathogenic *Spiroplasma* include *S. melliferum* in bees, *S. eriocheiris* in crayfish, and *S. citri* and *S. kunkelii*, which cause citrus stubborn and corn stunt disease, respectively, in multiple crops, and are vectored by leafhoppers.

Vertical transmission has evolved independently in *Spiroplasma* numerous times (Haselkorn et al., 2009), and in a wide range of arthropod orders. Two distantly related lineages, the ixodetis and poulsonii groups, have been studied the most, and have been shown to kill males in beetles (Hurst et al., 1999), flies (Poulson & Sakaguchi, 1961), lacewings (Hayashi et al., 2018), and butterflies (Jiggins et al., 2000), and to protect *Drosophila* flies from parasitic nematodes and wasps (Jaenike et al., 2010; Xie et al., 2010), and pea aphids from pathogenic fungi (Lukasik et al., 2013). In addition to ovarian tissue, vertically transmitted *Spiroplasma* are abundant in hemolymph, where they are extracellular. It is therefore easier to establish novel infections of many inherited *Spiroplasma*, as hemolymph can be transferred from infected to uninfected hosts (Sakaguchi & Poulson, 1963). By feeding on infected hemolymph, ectoparasitic mites were shown to transfer *Spiroplasma* between species, acting like a dirty syringe (Jaenike et al., 2007).

Other symbionts

Beyond the five major lineages of facultative inherited symbionts of insects, there are many other inherited bacteria. For example, the alphaproteobacterium *Lariskella* is widespread in stinkbugs and ticks, but its biology is completely unknown (Matsuura et al., 2012). Three

gammaproteobacterial lineages likely infect a wide range of insects but have been little studied as symbionts. While members of the genus *Rickettsiella* are well-known pathogens of insects and crustaceans (Duron et al., 2018), a number are either confirmed or likely vertically transmitted symbionts in aphids (Tsuchida et al., 2010), leafhoppers (Iasur-Kruh et al., 2013), and spiders (Zhang et al., 2018a, b), and this is likely to be just the tip of the iceberg (Duron et al., 2018). Primers are available for multi-locus sequence typing of *Rickettsiella* (Leclerque et al., 2011), so more widespread screens and phylogenetic characterisation should be relatively straightforward. The only inherited *Rickettsiella* symbiont that has been studied in any detail infects pea aphids, causing them to change in colour from red to green (hence its name *R. viridis*) (Tsuchida et al., 2010), resulting in reduced predation by ladybird beetles (Polin et al., 2015). *Rickettsiella* infection also protects aphids against pathogenic fungi (Lukasik et al., 2013).

Within the Gammaproteobacteria, the Enterobacteriaceae has repeatedly given rise to many insect symbionts, and they can be difficult to discriminate because they are often closely related to common host-associated bacteria. At least two lineages of enteric bacteria are likely widespread symbionts, although it will be challenging to discriminate between gut symbionts and intracellular inherited ones. Facultative *Sodalis* infections have been found in parasitic and social Hymenoptera (Betelman et al., 2017; Rubin et al., 2018), lacewings (Sontowski et al., 2020), and in tsetse flies. Interestingly, many *Sodalis* strains have repeatedly evolved into obligate symbionts, in many Hemiptera, weevils, lice, and louse flies (Santos-Garcia et al., 2017). A clade allied with *Pectobacterium* includes facultative symbionts of bedbugs (Hypsa & Aksoy, 1997) and leafhoppers (Campbell & Purcell, 1993), and obligate symbionts of mealybugs (Husnik & McCutcheon, 2016) and lygaeids (Kuechler et al., 2011). More host-restricted enteric symbionts include the well-studied sister clades *Hamiltonella* and *Regiella* that defend aphids against parasitic wasps and pathogenic fungi (Oliver et al., 2003; Scarborough et al., 2005; Vorburger et al., 2010); *Hamiltonella* is also an obligate symbiont in some *Bemisia tabaci* whiteflies (Rao et al., 2015).

Work on inherited bacterial symbionts of insects has eclipsed that on eukaryotic and viral symbionts. While a number of insects have evolved obligate inherited nutritional symbioses with fungi (e.g., Fukatsu & Ishikawa, 1992; Gomez-Polo et al., 2017; Matsuura et al., 2018), facultative fungal symbionts have been largely overlooked (Gibson & Hunter, 2010). As one example, the cockroach parasitoid *Comperia merceti* hosts a vertically transmitted ascomycete that reduces host fitness in the laboratory and is thought to persist only due to horizontal transmission (Gibson & Hunter, 2009). Microsporidia are common intracellular parasites of insects and are also a likely source of inherited symbioses. Indeed, feminising microsporidia have evolved independently numerous times in amphipods (Terry et al., 2004) but have not yet been documented in insects. Finally, there are a few documented cases of inherited viruses that are male-killers, in the tea tortrix moth *Homona magnanima* (Nakanishi et al., 2008) and in the fruit fly *Drosophila biauraria* (Kageyama et al., 2017). Viruses with at least some vertical transmission in their life cycle are ubiquitous (e.g., O'Neill et al., 1995; Longdon & Jiggins, 2012; Xu et al., 2014), and uncovering their host effects without a striking phenotype such as male-killing will be very challenging.

6.5.4 Suggestions and Potential Pitfalls for Entomologists Studying Inherited Symbionts for the First Time

In this final section, we briefly provide some suggestions for researchers who are studying insect parasitoids and predators but who have not previously worked on insect symbionts, on some first steps and potential pitfalls in beginning to characterise inherited symbionts.

The first step in characterising the inherited symbionts in one's favourite insect study

organism will involve screening samples from the wild and/or laboratory colonies, using both targeted and untargeted approaches. Targeted approaches involve amplifying symbiont DNA using polymerase chain reaction (PCR) with symbiont-specific primers; a wide range of primers are available that target the main lineages of facultative symbionts (e.g., Duron et al., 2008a, b; Russell et al., 2013), as well as others. It is essential to confirm that there has not been spurious DNA amplification, by Sanger sequencing some of the PCR products. Untargeted amplicon sequencing, using conserved 'universal' bacterial 16S ribosomal RNA primers and PCR, is also very useful for identifying potentially interesting bacterial symbionts. However, this approach will amplify (many) bacteria that are not intimate insect associates, as well as gut and inherited symbionts, and it is recommended to sample different parts of the insect's body (for example, removing the gut), and to include negative controls and sufficient numbers of replicates. Bacteria with divergent 16S rRNA sequences, that are found at relatively low titres, or that have restricted distributions in the body (for example, in bacteriomes), have the potential to be missed in these surveys. To screen for fungal symbionts, one can use untargeted amplicon sequencing of fungal 18S rRNA, or fungal-specific PCR primers (e.g., Fukatsu and Ishikawa, 1995), although ribosomal and mitochondrial genes of many fungal insect symbionts contain large insertions of selfish genetic elements, such as mobile introns, and can be therefore challenging to amplify. Putative symbiotic viruses will be the most challenging to identify, as they evolve so rapidly and are so diverse that there are not conserved primers to amplify them, and metatranscriptome or metagenome sequencing is necessary (e.g., Webster et al., 2015).

Once a potentially interesting symbiont has been identified, a straightforward next step is to determine its prevalence in the field, using PCR with symbiont-specific primers. This will give clues as to the role the symbiont may be playing. For example, sex-ratio distorters will be absent or rare in males, and symbionts that induce strong cytoplasmic incompatibility are expected to occur at high prevalence. At the same time, it is also very useful to obtain insect mitochondrial sequence, as insect mitochondria are inherited maternally. Symbionts or symbiont haplotypes that are perfectly associated with mitochondrial haplotypes provide strong evidence that the symbiont is vertically transmitted (Hurst & Jiggins, 2005). If a symbiont infection is found to be only associated with one mitochondrial haplotype and with little sequence variation, then it might suggest that the symbiont has recently spread (e.g., Jaenike et al., 2010; Himler et al., 2011), for example in association with cytoplasmic incompatibility (Turelli et al., 1992).

Sequencing multiple genes will help determine the symbiont's phylogenetic affinities. 16S rRNA sequence is not sufficient as it (usually) evolves too slowly and is not variable enough, and the commonly used *Wolbachia* wsp gene experiences extensive recombination (Werren & Bartos, 2001); sequencing multiple genes is now standard and there are multiple primers and multi-locus sequence typing approaches available for many symbiont lineages (Baldo et al., 2006; Haselkorn et al., 2009; Weinert et al., 2009; Duron et al., 2010). Sequencing multiple genes will also help identify symbionts that may lie on an interesting branch of the phylogenetic tree, and that should be targeted for whole-genome sequencing. In fact, the costs of sequencing have dropped such that it is feasible to generate complete or partial genomes from an interesting symbiont using next-generation sequencing approaches, and we recommend reaching out and establishing collaborations with researchers with expertise on the genomics of specific symbionts.

There are two major sources of false positives when screening for symbionts. First, there is the possibility of amplifying DNA from symbionts of prey, parasites, or endoparasitoids. There are a number of ways to get around this, including sampling different parts of the body (Goodacre et al., 2006), such as the head, legs, or ovaries, or rearing insects in the laboratory, or bringing them into the laboratory and only extracting DNA after they have not had any food for a few days. Another possible source of contamination is

symbiont DNA that has become incorporated in the host genome via lateral gene transfer. There are many examples of this, especially involving *Wolbachia* (Kondo et al., 2002; Hotopp et al., 2007), and this is perhaps not surprising since facultative inherited symbionts are so abundant in germline, and since so many insects harbour them. Sometimes entire or almost entire symbiont chromosomes are integrated, and these integrations can be fixed or polymorphic. For example, a recent genomic survey of 200 wild *D. melanogaster* lines found that four had large germline insertions of *Wolbachia* (Huang et al., 2014). Integrated symbiont DNA is often non-functional and pseudogenised, although a recent study found that ~ 80% of the genome of a feminising strain of *Wolbachia* has integrated into a pillbug genome, where it now acts as a female-determining sex chromosome (Leclercq et al., 2016). There are a number of ways to potentially rule out horizontal transfer, including sequencing multiple genes and confirming that they have not become pseudogenised (e.g., Morrow et al., 2015). Also, inserted sequences will not be eliminated following antibiotic treatment.

As well as false positives, one should also be mindful of false negatives. These can occur for a number of reasons, including primer mismatches and difficulties in extracting DNA, for example due to thick cell or spore walls. Some symbionts have been reported to be found at very low titres in their hosts (e.g., Arthofer et al., 2009), which makes it more challenging to successfully amplify their DNA. Low-titre infections may still have important impacts on their hosts. For example, a beautiful (and cautionary) study recently reported an interesting low-titre strain of *Wolbachia* in *Drosophila pseudotakahashii* (Richardson et al., 2019). This *Wolbachia* is often absent or found at low titre in the adult sons of infected females, yet still causes strong CI (i.e., when the sons of infected females mate with uninfected females).

Finally, one should be mindful about multiple infections. Many insects host multiple inherited symbionts (e.g., Gueguen et al., 2010; Toju & Fukatsu, 2011; Russell et al., 2012) and/or multiple strains of the same symbiont; the latter is especially common in CI *Wolbachia*, with coinfecting strains that are often mutually incompatible (Perrot-Minnot et al., 1996; Duron et al., 2006). So one should be careful not to ascribe an effect due to a symbiont without ruling out the presence of others. We wonder whether some phenotypes have been inadvertently attributed to *Wolbachia*, because it is so common and has been studied in more depth and often before other symbionts were known or appreciated, and because antibiotic treatment may remove all symbionts. Finally, the presence of multiple symbionts can affect host fitness and/or reproductive manipulation. For example, *Sogatella furcifera* planthoppers infected with both *Wolbachia* and *Cardinium* induce much stronger CI than insects infected with either symbiont alone (Nakamura et al., 2012).

Of course, once one has identified a symbiont infection, the next steps are to understand how it affects its host and how it is transmitted, in the laboratory and in the field. This means that it is important to establish symbiont-free insect lines as controls, either by antibiotic or heat treatment (Li et al., 2014), or by isolating a naturally uninfected line. Care should also be taken to ensure that the lines have similar nuclear genetic backgrounds and gut microbiota, and that there are not residual effects of antibiotic treatment (e.g., Ballard & Melvin, 2007).

6.5.5 Conclusion

It is a very exciting time to study facultative inherited symbionts, and the field is changing rapidly, with new approaches and developments, from both applied and basic research perspectives. Genomics has revolutionised the study of insect symbionts. Other recent breakthroughs have included the ability to culture symbionts (e.g., Brandt et al., 2017; Masson et al., 2018), and to express symbiont genes in flies and yeast, in order to understand their function (Sheehan et al., 2016; Lepage et al., 2017; Harumoto & Lemaitre, 2018). Even though so many exciting discoveries have been made, many new symbiont

lineages almost certainly await discovery. Further, we do not understand how most of the symbionts we already know about persist in their hosts. Indeed, many of the bottlenecks in terms of understanding insect–symbiont interactions are ecological, and this is especially relevant in terms of symbionts that affect insect natural enemies. How do they move through food webs? How important are they in affecting host defence, host choice, host range, host and prey suitability? Entomologists and insect ecologists ignore symbionts at their peril.

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Population Dynamics

7

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7.1 Introduction

Predators and parasitoids are important components of all insect communities and are therefore of central interest to ecologists studying the complex factors driving the dynamics of species interactions and community structure. Knowledge gained from studies of predator and parasitoid populations is also of immense practical value in insect pest management (Hassell & Waage, 1984; Murdoch et al., 1985; DeBach and Rosen, 1991; Van Driesche et al., 2010; Heimpel & Mills, 2017; Hajek & Eilenberg, 2018; McEvoy, 2018; Segoli et al., 2023).

In this chapter, we aim to demonstrate how ecologists and biological pest control researchers can assess the role of natural enemies in insect population dynamics, and how the information obtained can be put to use in biological control. We begin by reviewing methods for demonstrating and quantifying predation and parasitism (Sect. 7.2). We then examine the different techniques for determining the effects of natural enemies on insect population dynamics empirically and through mathematical modeling (Sect. 7.3). Finally, we examine ways in which this and other information can be used in choosing appropriate biological control agents for introduction (Sect. 7.4).

7.2 Demonstrating and Quantifying Predation and Parasitism

7.2.1 Introduction

Most studies of pest control by predators and parasitoids examine pest and natural enemy presence and/or abundance and then qualitatively infer their impact. While this provides useful data to address a range of ecological questions, a quantitative measure of impact is critical for guiding pest management decision-making. For

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example, Mace and Mills (2017) argued that to encourage adoption of conservation biological control, metrics need to be developed that can predict current activity and future potential of biological control. They evaluated natural enemy metrics to explore how well they performed in predicting current and future biological control of the walnut aphid, *Chromaphis juglandicola*, in California walnut orchards. Some metrics based on direct measures of natural enemy activity, such as percent parasitism and predator–prey ratio, were effective indicators of current biological control activity. However, Mace and Mills (2017) highlighted that predicting future control through the season using natural enemy metrics can be misleading due to the confounding effect of within-year density dependence in the pest population.

Furlong and Zalucki (2010) reported that less than half the studies of lepidopteran pests and their natural enemies used methodologies that would allow measurement and objective assessment of the impact of natural enemies. Similarly, a meta-analysis of the response of pests and natural enemies to landscape complexity found only 13 of 46 studies included a measure of natural enemy impact (Chaplin-Kramer et al., 2011). Merely examining species presence and/or abundance and inferring impact means it is difficult to make informed pest control decisions incorporating natural enemy activity, as there is no quantitative evidence of impact. Direct estimation of natural enemy impact can provide a tangible metric for determining the point at which the impact no longer maintains populations of pests below economic damage thresholds (Macfadyen et al., 2015).

In this section we present techniques that can be applied to both field and laboratory populations of natural enemies and their prey (1) to demonstrate that natural enemies can have a significant impact upon host and prey populations, and (2) to quantify rates of predation and parasitism to provide indices of the impact of biological control of value to pest management.

7.2.2 Exclusion of Natural Enemies

Natural Enemy Exclusion

Exclusion methods, in which pest abundances are monitored in the absence and presence of natural enemies, are widely used to estimate the impact of predation and parasitism in the field. Suitably designed exclusion barriers coupled with careful non-destructive population sampling has been used, in combination with life-table construction, to effectively demonstrate the impact of predator and parasitoid complexes on pest populations under a range of conditions (Furlong et al., 2004b, 2008). The principle behind their use is to quantify natural enemy impact by comparing the growth in prey population in plots (any habitat unit, from part of a plant to a whole plant or a group of plants) from which natural enemies have been excluded with that in control plots to which natural enemies have free access. In the context of predation, although it is commonly assumed that prey missing in the field have been eaten by predators, this may not always be the case and Castellanos et al. (2015) have documented the bias that can result from non-consumptive effects of predation in exclusion experiments (Sect. 7.2.5).

Various exclusion techniques have been employed, including mesh cages placed over individual plants or groups of plants, mesh sleeve cages placed over branches or leaves, clip cages attached to leaves, greased plastic bands tied around tree branches and trunks, and vertical barriers, constructed of plastic or wood, around plants. The most appropriate technique will depend upon the natural enemies being investigated, and whether the aim is to exclude all natural enemies or to exclude particular species or groups of species. For example, a terylene mesh/gauze cage placed over a plant ought, if the mesh size is sufficiently small, to exclude all aerial and surface-dwelling insect natural enemies. By increasing the mesh size slightly, small parasitoid wasps may be allowed in, while increasing the mesh size further will allow larger

types of natural enemy to enter as well. Using a cage with its sides raised slightly above the ground allows ground-dwelling predators such as carabid beetles and ants to have access to aphids on cereals, while excluding adult hoverflies and flying parasitoids. Conversely, a trench or a barrier can prevent access to ground-dwelling predators but allow access to aerial predators and parasitoids. Barriers that exclude only a sub-set of the natural enemies that attack a pest can be used to illustrate the importance of specific enemy groups to biological control (Bográn et al., 1998; Medina and Barbosa, 2002; Gardiner and Landis, 2007; Xiao and Fadamiro, 2010; Martin et al., 2013; Rusch et al., 2013). When paired with population models, exclusion methods can provide valuable insight into the direct economic impact of certain biological control agents (Östman et al., 2003; Landis et al., 2008).

Exclusion barriers can be used to enclose already existing populations of prey, in which case the density of the prey at the start of the experiment will need to be estimated and recorded. Alternatively, exclusion barriers can be used to enclose plants or plant parts that were free of, or have been cleared of, prey and that can then be loaded with a fixed number of prey. The latter approach has the advantage that equivalent starting densities of prey/hosts can be used in both exclusion and control plots, and that the potential for immature stages of parasitoids being present within pre-existing hosts can be eliminated from the experimental plots. It may be necessary to use a systemic insecticide when eradicating prey such as leafhoppers or planthoppers from a plot, in order that any prey eggs present within plant tissues are killed. Of course, loading with prey cannot take place until one can be sure that the plant is free of the insecticide.

Exclusion studies need to account for both the effectiveness of the barrier and its effect on the survivorship or population growth of the focal herbivore. For example, mesh cages can alter microclimatic factors such as light intensity, humidity, and temperature. To account for this, exclusion cages that prevent all predators are sometimes paired with sham cages with a larger mesh size or cutouts allowing predator access (e.g., Costamagna et al., 2007). This approach has been used in several studies (Costamagna et al., 2008; Costamagna & Landis, 2011; Samaranayake & Costamagna, 2018). Medina and Barbosa (2002) used cages with varied mesh size along with sticky barriers to examine predation of large and small tussock moth (*Orgyia leucostigma*) larvae by flying invertebrates, crawling invertebrates, and birds. Large larvae were more frequently removed from cages allowing access by birds. However, the results for small larvae illustrated the importance of using adequate controls, as just as many small larvae disappeared from the treatment that excluded all predators as from the control treatment allowing access to all predators.

In order to separate the effects of microclimate and natural enemy exclusion upon prey populations, it is necessary to use exclusion techniques that are either: (1) as similar as possible in construction, or (2) very different in construction, but which nevertheless provide similar microclimatic conditions in their interiors. Kaser and Heimpel (2018) conducted an exclusion-cage experiment designed to isolate the impact of an accidentally introduced parasitoid of the soybean aphid (*Aphis glycines*) in North America from the other resident natural enemies of the soybean aphid. They designed five exclusion cages, including a sham cage. The sham cages were intended to simulate the microclimatic conditions of predator and total exclusion cages, but to allow natural enemies to enter in a manner similar to open cages. They found no significant differences between sham cages and open cages in aphid densities or aphid population growth rates; therefore, cage microclimate did not differentially affect birth and death rates of the aphids between treatments.

If the prey or hosts are mobile, both immigration and emigration may differ between exclusion and inclusion treatments, which can be a problem (Kindlmann et al., 2015). In order to rule out the possibility that aphid densities in fully caged cereal plots were augmented as a result of prevention of emigration of alatae, Chambers et al. (1983) removed all alate aphids that settled on the insides of some of the

experimental cages whilst allowing them to remain in other experimental cages. Removal of alatae was found to not alter the pattern of population change in the cages. Therefore, recolonisation of shoots inside experimental cages was unlikely to have been a cause of the difference observed in aphid densities between caged and open plots.

If prey densities increase in the exclusion plots, they may do so to such an extent that predator species (e.g., coccinellids, hoverflies) other than the ones that are excluded (e.g., carabid beetles) are preferentially attracted to the exclusion plots through their aggregative responses (Sect. 1.15.2). The impact of the excluded natural enemy species may thus be underestimated. This limitation applies particularly to the use of barriers and trenches, where the enclosed plants remain exposed to invasion by a variety of aerial predators. In addition, total exclusion of natural enemies is difficult to achieve and, consequently, it is important to check for the presence of natural enemies and to count them in the exclusion plots either during or at the end of an exclusion experiment. Exclusion methods employing barriers that are far from completely effective in excluding natural enemies are, strictly speaking, interference methods (see below).

Whilst exclusion methods can reveal that natural enemies have a significant impact upon prey populations, other methods generally need to be applied before the predator–prey interaction can be quantified. The results need to be related to the density of predators present in the habitat if realistic estimates of predation rates are to be obtained. Additionally, exclusion experiments provide minimal information, if any, on the dynamics of the predator–prey or parasitoid–host interaction, a limitation that applies also to several of the other methodologies described below. This problem can be at least partly overcome by the construction of paired life tables for the insects in exclusion and control plots (Van Driesche & Bellows, 1996; Itioka et al., 1997) (Sect 7.3.4).

Gardiner et al. (2009) used data from exclusion cages to develop a biological control services index (BSI) to quantify the extent of natural enemy control in crop fields, where:

$$BSI = \frac{\sum_{p=1}^{x} \frac{Ac,p - Ao,p}{Ac,p}}{x} \qquad (7.1)$$

Ac is the number or density of prey in each exclusion plot *p* a given number of days following the initiation of the experiment, *Ao* is the corresponding number or density of prey in each open plot *p* with access to natural enemies, and *x* is the number of replicate plots. Gardiner et al. (2009) found that BSI values for an invasive aphid, *Aphis glycines*, in soybean fields in north-central USA increased with landscape diversity. Similarly, Woltz et al. (2012) found BSI values to be high in soybean fields in Michigan, USA regardless of local habitat management or the diversity of the surrounding landscape.

Natural Enemy Interference

Although physical removal is considered a method of predator exclusion it is, as mentioned above, rarely completely effective and is thus better described as 'interference'. For large, relatively slow-moving predators it involves removal by hand while small, active predators and parasitoids can be removed using an aspirator. This method has advantages in that confounding microclimatic effects can be ruled out (since cages are not used), and the contribution of particular natural enemy species to parasitism and predation can be relatively easily assessed. However, the method also has the disadvantage that removal of natural enemies is very labour intensive. For the method to provide more than just a crude measure of natural enemy effectiveness, a 24-h per day watch needs to be kept on plants, and several observers need to be involved in removing insects. Additionally, removal of natural enemies may disturb prey and thereby increase prey emigration, and predators and parasitoids may have the opportunity to kill or parasitise hosts before they are detected and removed.

A related 'biological check' method of interference exploits the fact that honeydew-feeding ant species, when foraging for honeydew sources

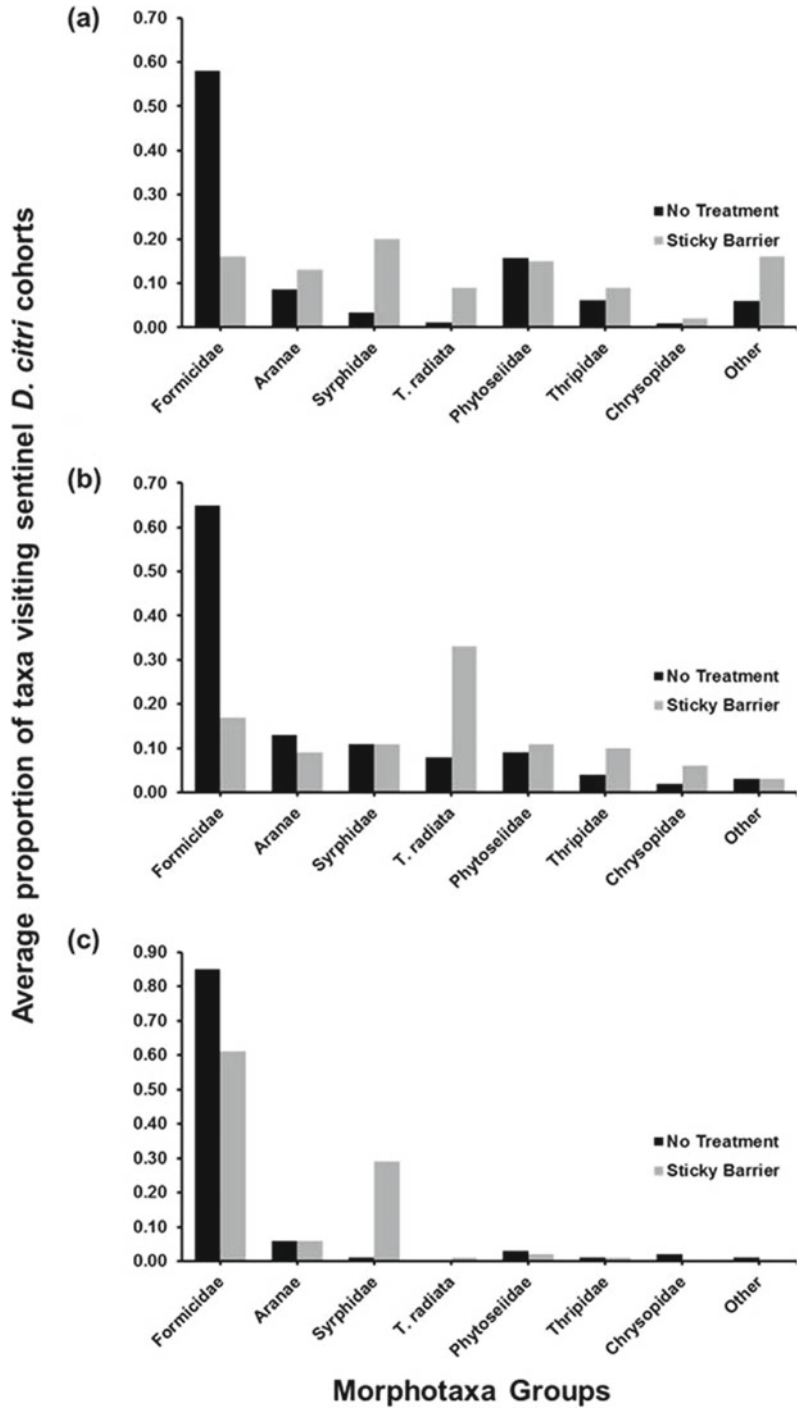

Fig. 7.1 The average proportion of taxa observed visiting sentinel *Diaphorina citri* cohorts under no and sticky barrier treatments at three localities. **a** Biocontrol Grove, **b** Lochmoor, and **c** Jurupa sites in Riverside County, California, USA. The "Other" category is comprised of uncommon predatory taxa including Coccinellidae, Anthocoridae, Mantidae, Forficulidae, and the brown garden snail, *Cornu aspersum* (from Kistner et al., 2017, with permission)

and tending homopteran prey, interfere with non-ant predators and parasitoids (Fig. 7.1), either causing them to disperse or killing them. In one set of plots, ants are allowed to forage over plants, whereas they are excluded from the other set. Natural enemies have access to both types of plot, but they are subject to interference by ants in the former. The method can be used with prey

that do not produce honeydew, provided either natural or artificial honeydew is made available to the ants. This method has several of the disadvantages of other interference and exclusion methods.

For the insecticidal interference method, test plots are treated with an insecticide, so as to eliminate the natural enemies, and the control plots are untreated. The insecticide used is either a selective one, or a broad-spectrum one that is applied in such a way as to be selective (e.g., reduced concentrations), affecting only the natural enemies. The main advantages of the method are that potential confounding effects of microclimate can be ruled out, and very large experimental plots can be used. As an alternative to blanket spraying of test plots, an insecticide trap method can be used. Ropes of plaited straw treated with insecticide, trenches dug in the soil and containing formalin solution or insecticide-soaked straw, or some other insecticide-impregnated barrier, can severely reduce the numbers of natural enemies entering test plots. Asiimwe et al. (2016) used this method to reduce natural enemies in treatment plots compared with unsprayed controls through applications of broad-spectrum insecticides, and to show that natural enemies exert a greater influence than plant quality on the seasonal dynamics of the whitefly pest *Bemisia tabaci* in cotton fields in Arizona, USA.

A limitation of insecticidal interference is that the numbers of prey may be inadvertently reduced due to the toxic effects of the insecticide (i.e., either the insecticide turns out not to be selective in action, or drift of a broad-spectrum insecticide has occurred) or they may be inadvertently increased due to some stimulatory, sublethal effect of the insecticide upon prey reproduction (e.g., prey fecundity may be increased). Insecticides can be tested in the laboratory for their possible sublethal effects upon prey reproduction. As for other interference methods (see above), total elimination of natural enemies from the test plots may not be achieved and so the full potential of natural enemies to reduce prey numbers is often underestimated. Additionally, limited information is provided on the dynamics of the predator–prey interaction, even where densities of natural enemies are known.

Combining Exclusion with Inclusion

One solution to the problem of achieving similar conditions in the different exclusion treatments is to carry out an exclusion/inclusion experiment. This involves the use of identical cages for the two inclusion treatments, an experimental treatment in which a known number of prey/hosts are added to the cage, and a control treatment in which a known number of predators and/or parasitoids as well as a known number of prey/hosts are added to the cage (e.g., Lingren et al., 1968; Rusch et al., 2016). This type of experiment has the added advantage that the densities of natural enemies are more precisely known and that *per capita* predation and parasitism rates can be calculated provided the densities used reflect those normally recorded in the field (taking aggregative responses of the enemies into account; Dennis and Wratten, 1991). A major disadvantage of inclusion experiments is that the cages can severely restrict or prevent the dispersal of natural enemies. The long-distance searching behaviour of foraging predators and parasitoids, in response to kairomones, may also be interfered with.

7.2.3 Sentinel Prey and Hosts

One method of examining predation and parasitism by invertebrates is to actively manipulate prey/host availability by establishing patches of sentinel prey/hosts and recording the rate of prey disappearance or accumulation of detectable traces of predation, or the rate of parasitism after a set period of exposure in the field. While the use of sentinel prey/hosts often includes 'non-natural' elements, such as inflated densities, non-natural distributions, and immobilisation of prey/hosts, which may distort the natural enemy–host interaction, it is suitable for comparative purposes.

Real, live or dead, sentinel prey/hosts in field experiments were initially used to measure

parasitism (Ôtake, 1967) or predation (Speight & Lawton, 1976) more than 50 years ago, and have been used productively since (e.g., Wratten & Pearson, 1982; Perez-Alvarez et al., 2019). However, several studies have documented important differences in predation between live and dead, and mobile and immobile prey. For example, the probability of removal of immobilised prey may be higher than for unmanipulated prey that are able to escape or defend themselves (Zou et al., 2017). Natural enemies may also have a preference for either mobile or immobile prey (Nagy et al., 2020). For example, Brooks et al. (2009) found higher predation of live, mobile prey than of dead, immobilised prey in a freshwater macroinvertebrate system, while Steward et al. (1988) reported that predatory wasps (Vespidae) preferred pinned to unpinned caterpillar prey. Hence, the method of prey manipulation can affect the estimation of predation rates.

A further important aspect of determining the validity of the sentinel method is to assess whether the predator of immobilised prey also consumes the prey in unmanipulated settings. Direct observation (Sect. 7.2.4) can provide first-hand information about the predators involved in pest suppression (Pfannenstiel & Yeargan, 2002; Westerman et al., 2003), but this method is laborious and less practical at night or under adverse weather conditions. Video recording of exposed prey can resolve these limitations as it allows continuous monitoring for extended periods under a wide range of environmental conditions (Frank et al., 2007; Grieshop et al., 2012; Nurdiansyah et al., 2016; Zou et al., 2017; Perez-Alvarez et al., 2019).

Predation can also be assessed using artificial prey. A rather superficial similarity to real prey is often sufficient to attract predators, though such artificial prey cannot move, defend themselves, or behave as true prey would, and the absence of chemical cues may conceal prey identity (Howe et al., 2009; Lövei & Ferrante, 2017). Artificial sentinel prey was first used by Edmunds and Dewhirst (1994) and have since been used successfully to quantify predator impacts against caterpillars in the field (e.g., Seifert et al., 2015, 2016; Clayborn & Koptur, 2017). Although artificial sentinel prey is less natural, traces of predation left by different predators are sometimes identifiable, making them suitable for comparative studies and the partitioning of total predation pressure by predator types (Lövei & Ferrante, 2017). Artificial sentinel prey is also cheaper to use than live prey, do not require rearing, can be simple to produce (as in the case of caterpillar models; Howe et al., 2009), can be standardised across sites (Roslin et al., 2017), and their density and distribution can be easy manipulated. For these reasons, the artificial sentinel method has been recommended for obtaining quantitative estimates of predation as an ecosystem service under field conditions (Meyer et al., 2015). However, it can only be used for generalist predators and not for specialist predators or for parasitoids.

While sentinel prey is often used to quantify predation, hosts can also be placed in the field for a set exposure period to estimate the impact of parasitoids (e.g., Letourneau et al., 2012; Thomson & Hoffmann, 2013). This is most commonly used for studies of egg (e.g., Keller & Lewis, 1985; Glenn & Hoffmann, 1997) and pupal parasitoids (Geden et al., 2020; Nieto et al., 2021), but can also be used for larval parasitoids (Todd et al., 2018; Rutledge et al., 2021). This allows comparisons of standardised parasitism rates across different crop types, at different times throughout the season, and across multiple habitats in agricultural landscapes (e.g., Thomson & Hoffmann, 2013; Macfadyen et al., 2015).

7.2.4 Direct Field Observation

Predation and parasitism can be observed directly in the field, which is valuable to identify relevant species interactions (Rosenheim et al., 1999), and to understand and quantify searching behaviour (Waage, 1983; Schenk and Bacher, 2002; Brechbuhl et al., 2010) and prey defence (Nelson, 2007). When sufficient observations are made it can also be used to quantify rates of predation or parasitism (van Nouhuys & Ehrnsten, 2004; Costamagna and Landis, 2007;

Latham and Mills, 2010; Naranjo & Ellsworth, 2017). Increasingly, video is being used for observation of relatively sedentary prey. This is efficient because multiple videos can be viewed by researchers at high speed, allowing systematic data collection and observation of even infrequent events. Additionally, infrared cameras can be used to record activities at night. Video cameras are generally less disruptive than human observation of natural interactions, though the installation of cameras can still be disruptive (Grieshop et al., 2012; Hemerik et al., 2018).

The Asian citrus psyllid, *Diaphorina citri*, is an economic pest of citrus because it vectors a bacterium that causes the lethal citrus disease huanglongbing. Kistner et al. (2017) studied predation and parasitism of *D. citri* colonies with and without access for ant mutualists in urban citrus. Based on a total of 19,200 h of video they were able to identify the natural enemy community and to show that when ants were excluded by a sticky barrier, visitation by syrphids, which are key predators, and the imported psyllid parasitoid, *Tamarixia radiata*, increased (Fig. 7.1).

Relatively few observational field studies include data that is sufficiently extensive and systematic to be used quantitatively. Those that do quantify the rates of predation or parasitism use many different approaches. As a classic example, Kiritani et al. (1972) estimated that, depending on season and leafhopper instar, between 10 and 63% of rice leafhoppers in a field are eaten by spiders, by estimating the number (n) of rice leafhoppers killed by spiders per rice hill per day as follows:

$$n = FC/P \tag{7.2}$$

where F is the number of predators seen feeding per rice hill during the observation period, C is the number of hours in a day that predators are actively feeding, and P is the average time, in hours, taken to eat a prey individual. Also studying spiders, Sunderland et al. (1986) quantified predation of aphids by web-spinning spiders based on:

$$n = prk \tag{7.3}$$

where n is the number of aphids killed/m^2/day, p is the proportion of ground covered by webs, r is the number of aphids falling from plants per m^2/day, and k is the proportion of aphids entering webs that are killed (determined from field observations and laboratory experiments). Using this approach, Sunderland et al. (1986) estimated that aphid populations could be reduced by up to 40% by spider predation.

7.2.5 Non-consumptive Effects of Predators and Parasitoids

Although the direct effects of predation and parasitism on prey abundance are critical to understanding population and community dynamics, indirect effects through prey responses that reduce the risk of predation and parasitism can also play an important role (Sih, 1986). Responses of insect prey to the threat of predation and parasitism include changes in behaviour (Ballantyne and Willmer, 2012; Siepielski et al., 2014) , life-history (Elliott et al., 2015; Sitvarin et al., 2015; Xiong et al., 2015), and physiology (Thaler et al., 2012; Rendon et al., 2016). Natural enemy-mediated changes in prey traits that do not involve direct consumption are termed non-consumptive effects (NCEs), risk effects or trait-mediated interactions (Hermann & Landis, 2017).

The majority of studies to date link NCEs to changes in behaviour, including changes in feeding (Rypstra & Buddle, 2013; Thaler et al., 2014), oviposition (Wasserberg et al., 2013; Sendoya et al., 2015), colonisation or dispersal (Ninkovic et al., 2013; Bucher et al., 2015; Kersch-Becker and Thaler, 2015), host-plant preference or habitat use (Wilson & Leather,

2012; Sidhu & Wilson Rankin, 2016) and increased predator avoidance (Hoefler et al., 2012; Lee et al., 2014). In general, prey tend to respond to natural enemies behaviourally to become less apparent and reduce encounters, which can often lead to a reduction in fitness due to reduced food intake and reproductive success.

The non-consumptive effects on prey from natural enemies can be quantified by manipulation of predator mouthparts (physical removal or gluing them shut), physical isolation of natural enemies from hosts using a barrier, or by isolation of individual natural enemy cues (such as visual or chemical cues). Such approaches allow different mechanisms of natural enemy detection to be studied and their impacts on prey fitness quantified (Hermann & Landis, 2017).

NCEs of natural enemies can be stronger than their consumptive effects and can have indirect effects that act at the ecosystem level (Preisser et al., 2005; Creel & Christianson, 2008; Buck et al., 2018). For example, Fill et al. (2012) studied the NCEs of an aphid parasitoid *Aphidius colemani* on both *Myzus persicae*, a host aphid, and *Acyrthosiphon pisum*, a non-host aphid. They found that the parasitoid reduced the population growth rate of the non-host aphid, probably through direct encounters while foraging for the host aphid, which caused the non-host aphid to drop from its host plant in response to the risk of attack. Thus, even specialist natural enemies have the potential to cause non-target impacts on insect herbivores in the broader community via NCEs. Similarly, Ingerslew and Finke (2017) extended this same study system to include a second aphid parasitoid *Aphidius ervi* that only parasitises *A. pisum*. The outcome for aphid suppression was influenced by interference in the consumptive effects of the two parasitoids, but also by additive contributions from both parasitoids to their NCEs. This illustrates that NCEs can arise from responses to both enemy and non-enemy species, adding further to the complexity of quantifying the impacts of predation and parasitism.

7.2.6 Molecular Approches for Determining Species Identities and Trophic Relationships in the Field

Electrophoresis was among the first molecular approaches used to quantify predation by fluid-feeding arthropod predators (reviewed by Solomon et al., 1996). However, this technique has been superseded by more sensitive methods of molecular gut content analysis (MGCA) that include both serological and DNA-based techniques (Symondson, 2002). Immunoassays using polyclonal antisera plus enzyme-linked immunosorbent assay (ELISA) have been used extensively for predator gut content analysis. For example, Sunderland et al. (1987) used this approach to compare different polyphagous predator species and the detectability of cereal aphid proteins in their gut contents. They found that antibodies to aphid proteins could be detected in the gut of a predator for relatively long time periods, and that 'maximum detectability times' were longer for spiders and staphylinid beetles than for some other predators. Two key problems with use of polyclonal antisera were a lack of reproducibility and a tendency to cross-react with proteins from other prey species (Symondson, 2002). An alternative monoclonal antibody-ELISA approach has been developed to overcome these limitations but has not been extensively used due to the extensive time and high cost associated with production of a suitable clone. The specificity of monoclonal antibodies can extend to detection of different life stages of conspecific prey in predator gut contents. It was used by Sigsgaard et al. (2002) to investigate cannibalism in the corn earworm, *Helicoverpa zea*. The related technique of immunomarking, in which prey items are marked with a generic immunoglobulin G (IgG) that can be detected in the gut contents of predators using an IgG-specific ELISA, has the distinct advantage that it avoids the need for development of prey-specific monoclonal antibodies (Hagler, 2006; Hagler et al., 2018). Prey items marked in

this manner can also be used to identify cannibalism and to determine whether the gut contents of predators represent true predation events or result of scavenging and the consumption of carrion.

Following the demonstration that shorter DNA sequences increased the amount of time that prey are detectable in predator guts, and that detectability was improved by using primers that amplified sequences from multiple copy DNA (Zaidi et al., 1999), drastic advances were made in the development of DNA-based detection techniques (Furlong, 2015). DNA markers have become the most widely used approach for the analysis of trophic interactions, with conventional and multiplex polymerase chain reaction (PCR) approaches based on specific primers being sufficient for detection of parasitism and predation by known natural enemy species, and next-generation sequencing based on universal primers providing an opportunity to investigate more complex species interactions that might include unknown species (Gonzalez-Chang et al., 2016; Schmidt et al., 2021).

Polymerase chain reaction primers have not only been used for quantifying predation, but also to provide accurate estimates of parasitism rates and identification of immature parasitoids dissected from hosts (Jones et al., 2005). In addition, as secondary parasitoids can be difficult to identify using morphological characters, Chen et al. (2006) developed specific primers for identification of two secondary parasitoids of *Lysiphlebus testaceipes*, a common generalist parasitoid of aphids. More recently, DNA metabarcoding (Sect. 3.2.2) has also been used to identify the primary parasitoids of the millet head miner *Heliocheilus albipunctella* in Senegal, where it was used as a viable alternative to host rearing for estimating rate of parasitism (Sow et al., 2019). Parasitoid richness and parasitism rates at four field sites were consistently higher for DNA metabarcoding than for host rearing, indicating that this technique shows promise for quantifying the importance and complexity of host–parasitoid interactions in the field. Liang et al. (2018) also developed a reliable and robust molecular technique to characterise the competitive interaction between two parasitoids *Diachasmimorpha longicaudata* and *Fopius arisanus* of the oriental fruit fly *Bactrocera dorsalis*.

Recent developments in the MGCA of predators include the field application of real-time PCR to estimate the number of copies of prey DNA in predator guts (Zhang et al., 2007), quantitative PCR to determine prey consumption indices for different predator species (Lundgren et al., 2009), ligase detection reaction (LDR) PCR (Li et al., 2011), terminal restriction fragment length polymorphism (tRFLP) (Juen et al., 2012) and next-generation sequencing (NGS) techniques (Pinol et al., 2014) for the investigation of predator diet breadth and detection of multiple predation events. One challenge in the application of NGS for MGCA is the excessive amount of predator DNA relative to prey DNA produced using universal primers. A cost-effective method to enrich prey DNA without the need to block predator sequences in PCR amplification can be achieved through DNA extraction from the gut only, coupled with a long lysis time and size selection for low molecular weight DNA (Krehenwinkel et al., 2017, 2019). In parallel with these technological advances, protocols designed to maintain the integrity of field-collected predators for MGCA have been developed following the field testing of different predator collection methods (Greenstone et al., 2011, 2012).

While molecular approaches provide several advantages over classic methodologies (e.g., natural enemy exclusion and direct observation) for the identification of trophic linkages involving predators, these tools still lack the quantitative rigour that clearly links prey detection to the number of pests killed (Furlong, 2015). For example, Firlej et al. (2013) used large field cages to measure the impact of predation by Carabidae on soybean aphid populations and found that predators identified to be of importance from MGCA did not provide suppression of aphid populations in field cages. A key advantage of molecular approaches, however, is that they provide a more accurate assessment of the diet breadth of generalist predators.

Macfadyen et al. (2015) stated that predators are often described as "generalist" feeders that consume a wide range of prey despite a lack of evidence. Using a PCR approach to determine what generalist predators have recently eaten, it is now clear that some species are not as "generalist" as previously thought (Chapman et al., 2013). Similarly, DNA-based approaches have the advantage that they can effectively reveal interactions within natural enemy communities as well as interactions with a target pest. For example, Traugott et al. (2012) found that more than half of the parasitoid DNA detected in the gut contents of generalist predators stems from direct predation of adult parasitoids.

Molecular methods are used to study biological control interactions involving small and sometimes cryptic predators and parasitoids (reviewed by Symondson, 2002; Harwood and Obrycki, 2005; Gariepy et al., 2007; Harwood et al., 2009; Weber and Lundgren, 2009; Furlong, 2015; Schmidt et al., 2021). They have also proved to be a valuable approach for assessing the occurrence of intraguild predation in the field (Aebi et al., 2011; Schoeller et al., 2012; Traugott et al., 2012; Davey et al., 2013; Rondoni et al., 2015). Such techniques not only enable researchers to accurately identify interactions between natural enemies and host or prey insect pests in the field, but also to quantify, at least in the case of parasitoids, their contributions to pest control services. Both are critical elements for building successful and sustainable IPM strategies for crop pests and will undoubtedly benefit from further technological advances (Schmidt et al., 2021).

7.3 The Role of Natural Enemies in Insect Population Dynamics

7.3.1 Introduction

Having reviewed some of the methods by which insect mortality due to natural enemies can be quantified, we now turn to the task of assessing the significance of mortality, due to natural enemies, for the dynamics of hosts or prey populations. Mortality factors acting on an insect population can decrease insect population size or induce fluctuations in population sizes, and potentially contribute to the regulation of population size toward a stable equilibrium. Population regulation is directly relevant to the process of the pest control using natural enemies. For a factor, such as parasitism or predation, to regulate, the strength of its action must be dependent on the density of the population affected. That is, it needs to be prey density dependent, so that the effect of the predator or parasitoid is proportionally larger at high prey population densities and smaller at low prey densities (Fig. 7.2). Negative density dependence operates through negative feedback on population size. This is most often considered as due to host or prey mortality, but may also involve decreased reproductive rate, dispersal, and immigration. If the proportion of hosts parasitised varies with changing host density, either temporally or spatially, this can profoundly affect the dynamics of the species. Density-dependent factors can also affect average population sizes (Sects. 7.3.4 and 7.3.7) and can, under certain conditions, induce perturbations (Sect. 7.3.4).

We begin by addressing the pros and cons of using percentage parasitism estimates as a metric to assess the impact of parasitoids on host population dynamics (Sect. 7.3.2). We then discuss the simple technique of assessing the impact of natural enemies by assessing the correlation of

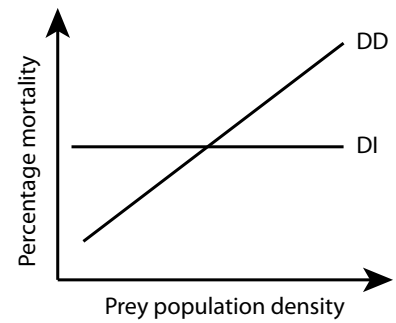

Fig. 7.2 A density-dependent mortality factor (DD) in which proportional prey mortality increases with population prey density and a density-independent factor (DI) in which proportional prey mortality is unrelated to prey population density

their numbers with those of the host populations (Sect. 7.3.3). We then review classical life-table analysis (Sect. 7.3.4) used to parse the contribution of each host stage and mortality factor to the dynamics of the host population. Next, we move to experimental rather than observational approaches to quantifying pest population control by natural enemies using manipulation and factorial experiments (Sect. 7.3.5). Following this, we discuss the influence of landscape fragmentation and metapopulations on natural enemy–host dynamics (Sect. 7.3.6) before reviewing the structure and stability properties of discrete-time and hybrid parasitoid–host models (Sect. 7.3.7) and closing the section with how to confront population models with field data (Sect. 7.3.8).

7.3.2 Percent Parasitism

The percent parasitism is the fraction of the host population that is observed to be parasitised. A high 'percent parasitism' of an insect pest population suggests that a parasitoid has a large impact on the host population size. While a high rate of parasitism will reduce the host population size, it does not necessarily reflect host population regulation, or long-term pest control.

First, the percent parasitism reported in a publication is the percent of a sample of the host population. It can either under- or over-estimate the impact of parasitoids on host population dynamics, depending on the size of a sample, the timing of the sample relative to the phenology of the species, and where the sample is taken from, relative to the distributions of both species. A life-table study of the host population (Sect. 7.3.4) can be used to assess a parasitoid's contribution to host population mortality locally. However, because it is not generally possible to obtain all of the information needed to make a life table, it is worthwhile to consider percent parasitism of a sample taking into account its limitations.

When samples are taken over time, say over a generation of a host, the percent parasitism will vary temporally depending on the phenologies of both species. Specific aspects of phenology that are relevant to how percent parasitism should be considered, such as whether generations are discrete or continuous, the length of time the host is susceptible, when the parasitoid is active during that time, and the development time of the parasitoid relative to the host (Godfray et al., 1994; van Nouhuys & Lei, 2004).

Phenological asynchrony of the host and parasitoid can be accounted for by measuring recruitment to both the host and the parasitoid (parasitised hosts) populations continuously. The ratio of total parasitoid recruitment to total host recruitment provides an unbiased estimate of total losses to parasitism. Another method uses the attack rate from field samples (Bellows et al., 1992). If individuals are collected at frequent intervals, reared under field temperatures, and the proportion dying from each cause recorded from one sample to the next, then the original percentage of the sample that was parasitised can be estimated. Gould et al. (1990) and Buonaccorsi and Elkinton (1990) provide equations for the calculations. The method requires that all hosts have entered the susceptible stage before the first sample and that no host recruitment occurs during the sampling period. Details and examples of these and other techniques for determining rate of parasitism can be found in Van Driesche and Bellows (1988), Furlong et al. (2004a), Toepfer and Kuhlmann (2006), Jenner et al. (2010) and Asiimwe et al. (2016).

The sampling method used will introduce a further error into the estimate of parasitism rate as methods are biased towards either parasitised or unparasitised hosts. In the above scenarios, samples are collected over time, generally covering the whole susceptible life stage of a host that has relatively discrete generations. In many instances, samples are taken less frequently or only once. If the sample is taken early, the percent parasitism for the host generation will likely be underestimated. If it is taken late, it may be overestimated. Similarly, rate of parasitism is generally spatially heterogeneous, at many scales (Hassell, 2000a; Segoli, 2016). Parasitism also varies among hosts in a population depending on what plant part they are on, the age of the plant and the plant species or variety (Kaiser et al.,

2017). For instance, Kishinevsky et al. (2016) found that parasitism of the whitefly *Bemisia tabaci* by *Encarsia* parasitoids varied among host plant species within a field, and was low on flowering host plant individuals. Parasitism rate also differs in different parts of a field (Ferguson et al., 2006) due to both local host density (Gunton & Pöyry, 2016) as well as location relative to field edges (Cronin, 2009), different parts of an agricultural landscape (Segoli et al., 2020), and in different types of landscapes (Marino & Landis, 1996; Tscharntke et al., 2007; Grab et al., 2018). Thus, sampling location, distribution of sampling points, and field site can either under- or over-estimate the rate of parasitism. Finally, the percent parasitism can underestimate the role of parasitoids for the dynamics of host populations if there are other forms of parasitoid-induced mortality. Host feeding (Jervis & Kidd, 1996; Zang & Liu, 2008; Emerick and Singh, 2016), unsuccessful parasitism and non-consumptive effects (Abram et al., 2019) contribute to host mortality and sometimes outweigh the effects of parasitism.

7.3.3 Correlation Methods

A useful indication of the impact of natural enemies can often be obtained by statistically correlating their numbers against those of their hosts. A high positive correlation may indicate a degree of prey specificity on the part of the natural enemy, that might reflect a rapid numerical response to variations in host density. Blubaugh et al. (2018), for example, found that the density of aphid parasitoids, primarily *Diaeretiella rapae*, was positively associated with aphid density on broccoli on farms with few lepidopteran pests (Fig. 7.3a). But on farms with many lepidopteran pests there was no association between parasitoid and aphid densities (Fig. 7.3b). They conducted an experiment and concluded that aphid parasitism was reduced by the presence of lepidopteran herbivores, presumably due to indirect effects of lepidopteran feeding influencing host plant cues used by foraging aphid parasitoids.

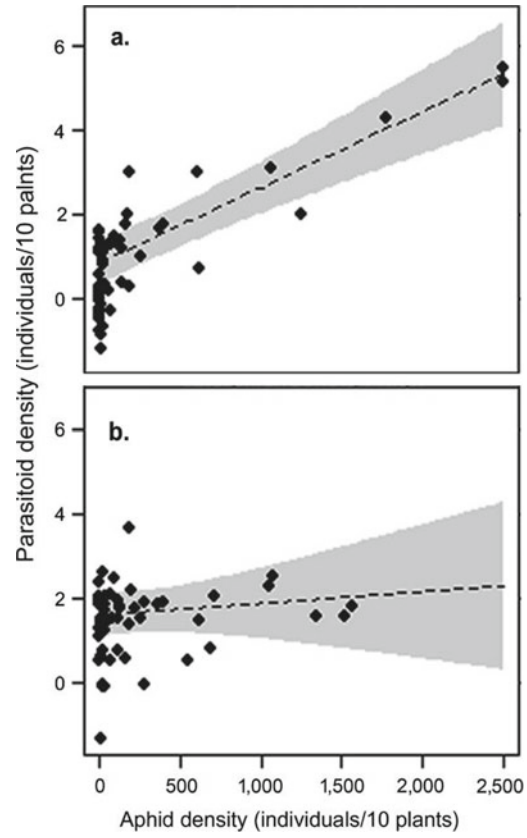

Fig. 7.3 Regression plots of a mixed-effects model of aphid densities and aphid parasitoid densities from a 2014–2015 survey of 52 organic farms where **a** lepidopteran herbivores rarely occurred (n = 54) and **b** where lepidopteran herbivores co-occurred with aphids at densities > 0.5 larvae/plant (n = 50). The dashed lines show the expected value of the linear regression, conditional on the random factors in the model, which were sample date and farm. The grey bands are 95% confidence intervals around expected values. The points are partial residuals, showing the association between aphid and parasitoid densities given the other factors in the model, sample date and farm (from Blubaugh et al., 2018, with permission)

Negative correlations, on the other hand, may indicate a slow or lagged numerical response by a predator to changing prey density. These responses are commonly shown by highly polyphagous predators that may 'switch' to feeding on a prey type only after it has increased in relative abundance in the environment. Negative correlations are also more likely to be associated with prey species that tend to show rapid changes

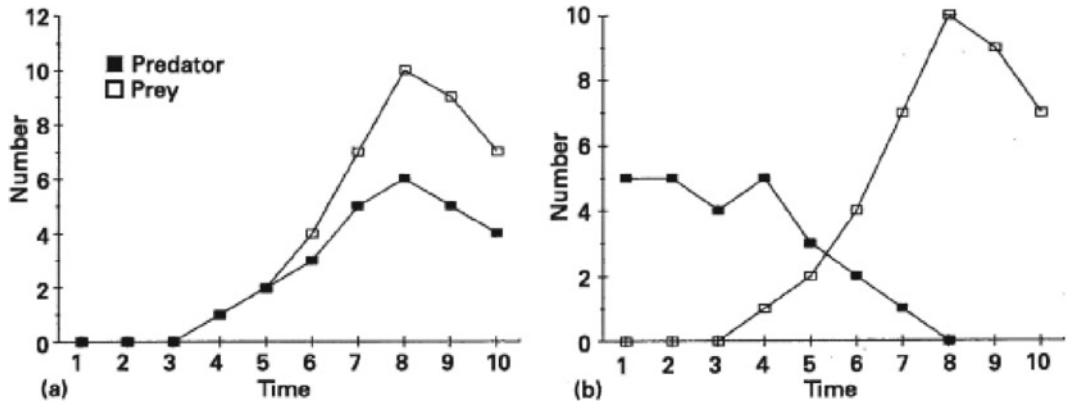

Fig. 7.4 Relationships between predator and prey population numbers which produce either positive or negative correlations: **a** a positive correlation between predator and prey numbers produced by a slow rate of prey increase coupled with a relatively low predator attack rate, such that prey numbers are not reduced, while predator numbers are still rising; **b** a negative correlation between predator and prey numbers caused by predators depressing prey numbers, which only increase after predator numbers have declined

in abundance, or with predators having a high attack rate (Fig. 7.4) (Murdoch, 1969).

Negative correlations are often found between aphids and their natural enemies because the aphids colonise a crop early in the season and increase rapidly. Enemies follow at different rates. For example, Bannerman et al. (2018) found a strong negative association between the soybean aphid (*Aphis glycines*) and its coccinellid predator *Harmonia axyridis* in soybean in Minnesota, USA. The coccinellid was absent early in the season as the aphid population increased. The population density of the aphid peaked at about seven weeks, and the coccinellid population peaked three weeks later, probably forcing the already declining aphid population to crash.

While in many cases natural enemies do cause host densities to decline, correlations can be created just as easily by predator populations tracking changes in prey numbers, rather than by bringing about those changes. Also, absence of a correlation should not be taken to imply that predators do not have any impact (Hassell & Waage, 1984). Therefore, conclusions based on correlation should be drawn with caution, and then only with an appreciation of the biology of the species involved and follow-up experiments.

7.3.4 Life-Table Analysis

Introduction

A life table shows, for each age, stage or time period, what the probability is that an individual will die before reaching the next age, stage or time period. It is especially useful in the study of insects, where developmental stages are discrete and mortality rates, and the causes of mortality, may vary widely from one lifecycle stage to another. Life tables are used to analyse the mortality of insect populations and determine key factors responsible for the pattern of change in total generation mortality within a population. They can further be used to determine how specific mortality factors, such as natural enemy species, affect prey or host population dynamics (Bellows et al., 1992). Below we show how types of processes relevant for pest control or population regulation can be identified and quantified using life tables. For instance, key lifestages for explaining population growth or decline under different conditions can be identified (e.g., Malabusini et al., 2022), density-dependent mortality can be distinguished from density-independent mortality, and delayed or over-compensating density dependence can be detected. Because some insect populations (e.g., aphids) tend to

have generations which overlap in time, while others do not, two quite different approaches have been developed for each category: the age-specific life table and the time-specific life table, respectively.

Age-specific Life Tables

The life-table approach was pioneered for insect populations in a study of *Drosophila melanogaster* by Pearl and Parker (1921). Varley and Gradwell (1960) extended the approach to discrete generations of the winter moth (*Operophtera brumata*) in the UK using key factor analysis, based on Haldane's (1992, reprinted from his 1949 publication) logarithmic method for comparing the contribution of successive mortality factors to total mortality (K). Varley and Gradwell's (1960) approach has some shortfalls (Royama, 1996) and in practice has been replaced by more powerful and sophisticated techniques focused on population growth rate rather than total mortality, and accommodating overlapping generations as well as sex (Brown et al., 1993; Sibly & Smith, 1998; Coulson et al., 2005; Chi et al., 2020). However, because the concept of key factor analysis, as presented by Varley and Gradwell (1960) , provides an intuitive way of quantifying and analysing the immediate cause of changes in population size, we illustrate it below.

The usefulness of Varley and Gradwell's (1960) approach depends on the availability of sequential life tables for several generations of a univoltine insect population. In temperate regions, for example, it is common for insect populations to overwinter as eggs and develop through a series of discrete stages in the spring and summer (Fig. 7.5). The adults then mature in the autumn to lay a new generation of overwintering eggs before dying. In this situation, generations remain separate. By obtaining population density estimates for the numbers entering each stage in the life-cycle, it is then possible to construct a composite life table from a sequence of life tables for each generation (Table 7.1). The numbers entering each stage can be estimated by direct assessment of recruitment (for example, by measuring fecundity or fertility), or by indirect calculation from counts of each stage. Several techniques are available which provide an estimate by the second route, and these are reviewed by Southwood and Henderson (2000).

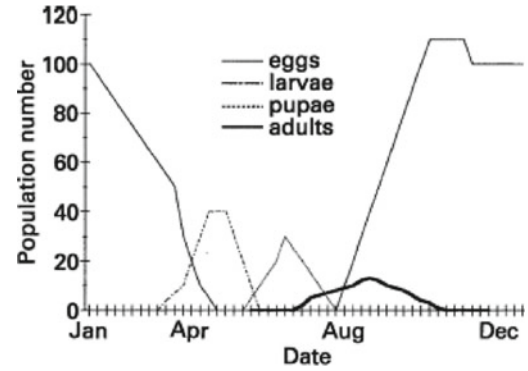

Fig. 7.5 Schematic life-cycle of a typical temperate zone univoltine insect population

Where stage mortalities can be partitioned into a number of definable causes, such as parasitism, predation and desiccation, they can be quantified separately in the table. In this way it may be possible to build similar life tables for particular natural enemies. By converting the numbers entering each stage in Table 7.1 to logarithms ($\log_{10}$), we can calculate for each successive mortality in any generation:

$$k = \log_{10}\text{number before mortality} - \log_{10}\text{number after mortality} \quad (7.4)$$

where k is a logarithmic measure of the proportion dying from the action of the mortality factor. Within each generation, we can thus determine a sequence of k-values, k_1, k_2, k_3, … k_n, corresponding to each successive mortality factor up to the adult stage. Mortality during the adult stage can be counted as one or more k-factors acting on the adults, or alternatively as a k-mortality acting on the next generation of eggs (Varley et al., 1973). The final post-reproductive mortality to act on a generation, i.e., that which brings generation numbers to zero, contributes nothing to between-generation variation in numbers and is not included in the analysis. The advantages of using k-values instead of

Table 7.1 Composite life tables for six generations of a hypothetical insect population with discrete generations. Each k-value is calculated as $k = (\log_{10}$ before mortality $- \log_{10}$ after mortality). $K = k_1 + k_2 + k_3$

Year	Eggs	k_1	Larvae	k_2	Pupae	k_3	Adults	K
1	1000	0.824	150	0.398	60	1.080	5	2.302
2	800	0.426	300	0.685	62	1.190	4	2.301
3	1200	0.681	250	0.455	50	0.824	12	1.960
4	700	0.942	50	0.204	50	0.699	10	1.845
5	500	0.553	140	0.301	70	0.766	12	1.620
6	1200	1.000	120	0.150	85	1.230	5	2.380

Note while such life tables have traditionally been presented in columns, putting them in rows, as is done here, makes spreadsheet regression calculations easier) (see also Fig. 7.6)

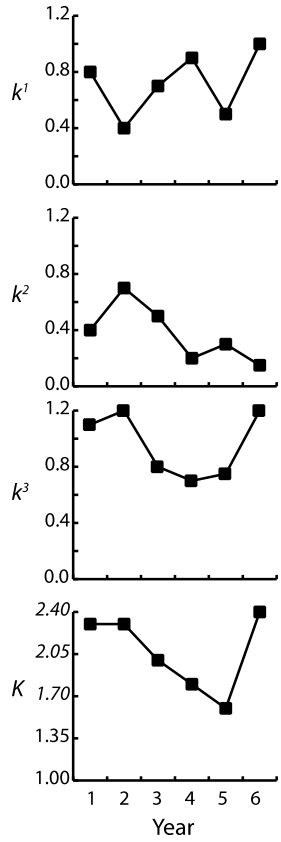

Fig. 7.6 Key factor analysis of the mortalities acting on a hypothetical insect population (see Table 7.1 for data)

percentage mortalities lie in the ease of calculation and the fact that k-values can be added together to give a measure for total generation mortality (K) (adding percentages would have no meaning because they would sum to well over 100%).

Plotting the k-values against generation may be enough to reveal the key factor(s) causing population change (Fig. 7.6). Here, variations in k_3 between generations most closely follow variations in overall mortality (K), indicating that k_3, is the key factor. Note that the key factor is not necessarily the factor causing greatest total mortality (k_1 in this case).

Detecting Density Dependence

Assessing which factors contribute to regulation of the population again involves plotting each k-value, this time against the $\log_{10}$ density on which it acts (i.e., before the mortality). In our example (Fig. 7.7) the plot of k_1 against log density of eggs contains six data points, corresponding to each generation. Similarly, k_2 is plotted against $\log_{10}$ density of new larvae, again with six data points, and so on. Positive relationships for any of these plots indicate that mortality is acting in a density-dependent fashion. A horizontal slope indicates density independence, while a negative slope indicates inverse density dependence. Regression analysis is generally used to calculate the significance of the slopes. Here, the only significant density dependence is found in k_2. However, the problem of statistical validity arises because as k-values are calculated in the first place from $\log_{10}$ densities, the two axes are not independent. Moreover, the independent variable ($\log_{10}$ density), estimated from population samples, is not error-free.

If density dependence is accepted, then the slope of the regression, b, can be taken as a measure of the strength of the density dependence. The closer b is to 1, the greater the stabilising effect of the mortality. A slope of $b = 1$

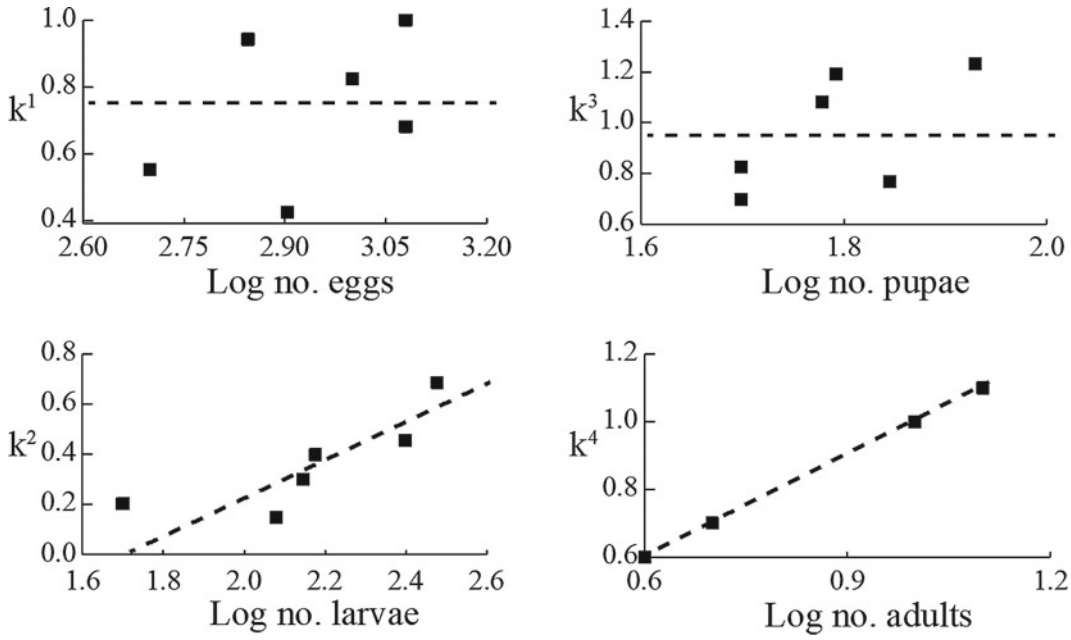

Fig. 7.7 The identification of density-dependent factors from life-table data. *k*-values for the different mortalities are plotted against the population densities on which they acted. In this case, only k_2 is significantly density-dependent ($k_2 = 0.86L–1.52$; $R^2 = 0.84$; $k_1 = 0.74$; $k_3, = 0.96$). k_4 is the last mortality to act, bringing numbers down to 0 (or in this case 1, which was used to make the log calculations workable). This remaining mortality is, by its nature, always density dependent but is not included in the analysis, as it contributes nothing to population variation or regulation

will compensate perfectly for any changes in density at this stage, while a slope of $b < 1$ will be unable to compensate completely for any changes (undercompensation). Slopes of $b > 1$ suggest overcompensation.

A Case Study: The Winter Moth

Varley and Gradwell's (1968, 1970) own study of the winter moth (*Operophtera brumata*), together with the various follow-up studies in England and Canada, are the best understood and most widely quoted examples of the use of age-specific life tables. We will briefly review some of the features of this study and use it to illustrate some of the potential problems in using key factor analysis.

The winter moth feeds on a wide range of mainly deciduous trees, and occasionally defoliates oaks. The life-cycle at Wytham Wood, near Oxford, UK, where the study was carried out, is as follows: eggs are laid in early winter in the tree canopy and hatch in spring to coincide with bud burst. The caterpillars feed on the foliage until fully grown, whereupon they descend to the forest floor on lines of silk and pupate in the soil. Adults emerge in November and December and females ascend the trees to mate and oviposit in crevices on the bark. There is, therefore, one generation each year (univoltinism).

Data collected between 1950 and 1962 reveal that 'winter disappearance' (k_1), during the period between the egg stage and that of the fully grown larvae, is the key factor explaining population variation between years. Parasitism, disease, and predation (k_2–k_6) are relatively insignificant in this respect (Fig. 7.8). The only significant regulating factor to be detected, however, was predation on pupae (k_5, Fig. 7.9), subsequently shown to be caused mainly by shrews and ground beetles (Frank, 1967; East, 1974; Kowalski, 1977). Parasitism showed no sign of being density dependent, either at the larval stage (k_2) or at the pupal stage (k_6), leading the authors to suggest that the wide variations in

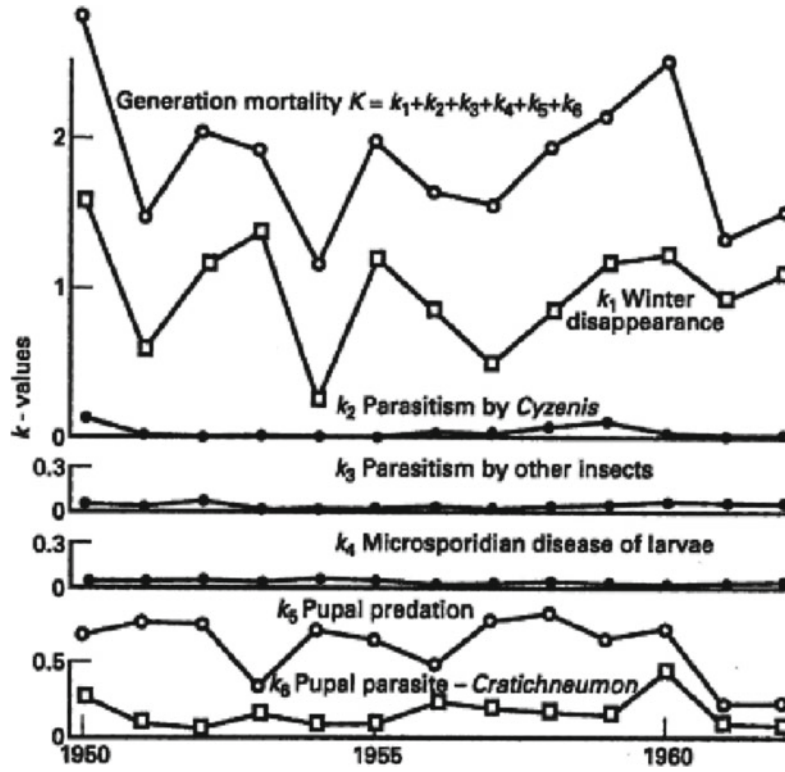

Fig. 7.8 Key factor analysis of the mortalities acting on the winter moth (from Varley et al., 1973, reproduced by permission of Blackwell Publishing)

densities from year to year, caused by the key factor 'winter disappearance', may be obscuring a possible delayed density-dependent relationship. The lack of any detectable regulating potential by the larval parasitoid *Cyzenis albicans* (k_2) was particularly surprising as this tachinid fly had been introduced in 1955 as a very effective biological control agent against winter moth in Nova Scotia, Canada (Roland & Embree, 1995). This difference could perhaps be explained by higher levels of *C. albicans* mortality in the UK. The parasitoid, although attacking the moth in the larval stage, continues to develop within the moth pupae throughout the summer and early winter and is therefore exposed to the same mortality factors as the moth pupae. Varley and Gradwell (1968, 1970) recorded as much as 98% mortality of *C. albicans* puparia. This is higher than that for winter moth pupae, but understandable as *C. albicans* spends 4 to 5 months longer in the soil, emerging in the spring.

Disadvantages of the Approach

The difficulty of obtaining sufficient field data highlights the single biggest problem of the approach, namely that of securing a long enough sequence of data to perform the analysis with a reasonable likelihood of detecting statistically significant relationships (Hassell et al., 1987). For insect populations having one generation a year, there is no guarantee of success with even a decade of data. This is especially a problem in the face of environmental change that alter processes affecting a species over the duration of the study. Moreover, the approach depends heavily on knowing all of the important factors to include in a study at the outset. There is not much scope for incorporation of new components at a later stage. There are several additional problems: (1) Several agents may act at the same time on a life-cycle stage, which can be accounted for using the marginal death rates which represent the proportion dying due to a factor in the absence of other independent factors that may act

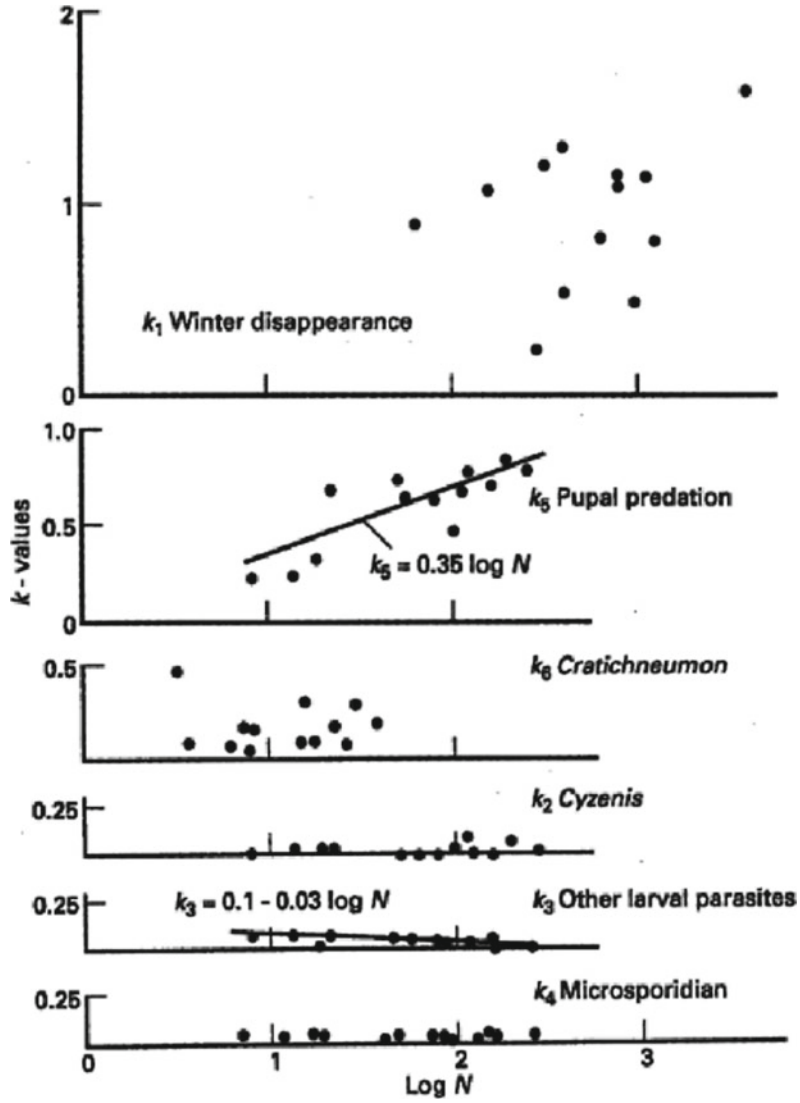

Fig. 7.9 *K*-values of the winter moth mortalities plotted against the population densities on which they acted. k_1, k_2, k_4 and k_6 are density independent; k_3 is weakly inversely density dependent; k_5 is strongly density dependent (from Varley et al., 1973, reproduced by permission of Blackwell Publishing)

at the same time (Elkinton et al., 1992). (2) Some of the mortality categories in the life table may contain, or mask, a number of others which could be important key or regulating factors. This is particularly likely to be the case with poorly understood, broad categories, such as 'winter disappearance' in the winter moth example. (3) Life-table analysis methods are based on correlation, so do not provide an unambiguous estimation of cause-and-effect relationships. (4) It is possible for strongly regulated populations to show little variation from equilibrium, and this may make statistical detection of the processes of regulation difficult using traditional life-table methods.

Time-Specific Life Tables

Time-specific (or vertical) life tables are suitable for use with populations in which the generations overlap, due to a short development time of the immature stages relative to the reproductive period of the adults (Kidd, 2010). At equilibrium, such species (such as humans and aphids) achieve a stable age distribution (Lotka, 1922) in which the proportion of the population in each age group or stage remains constant. In this

situation, all the ecological processes affecting the population are, at least in theory, operating concurrently. This means that the relative numbers in each age group at any instant in time provide an indication of the proportional mortality from one age group to the next. We cannot deduce from this what mortality factors are operating, or whether any regulation is occurring, so the value of a time-specific life table is limited in this respect.

Estimating mortality from parasitism is difficult when generations overlap. Van Driesche and Bellows (1988) provide an analytical method for doing this. Hughes (1962) developed a technique based on the time-specific life-table approach, which could be used for analysing aphid populations with a stable age (i.e., instar) distribution. Using a graphical method to compare population profiles at successive physiological time intervals, Hughes (1963) was able to partition the mortalities acting on the different instars, for example, parasitism, fungal disease and 'emigration'. As Hughes (1972) pointed out, there is, however, no easy way of estimating errors in the construction of these life-table diagrams. In fact, the technique is dependent on the assumption of a stable age distribution. Whilst Hughes' (1972) method is now considered to be of limited applicability, his work did lead to the development of the earliest simulation models for analysing insect populations with relatively complex population processes, such as density dependence (Knape and de Valpine, 2012; Andow & Kiritani, 2016). For field populations with overlapping or partially overlapping generations, the use of such models is now the only sensible way forward. These techniques are discussed in detail below (Sect. 7.3.8).

7.3.5 Manipulation and Factorial Experiments

The problems of detecting density dependence from life-table data have already been discussed (Sect. 7.3.4). One way of testing directly whether density-dependent mechanisms are operating is to carry out a 'convergence experiment' (Nicholson, 1957), in which subpopulations are manipulated to achieve artificially high or low densities and are then monitored through time (Harrison & Cappuccino, 1995). Convergence to a common density is then taken as evidence for density-dependent regulation. Practical difficulty in manipulating the density of some species, and knowledge of what densities are high or low, may limit the usefulness of this technique. Among successful studies, Brunsting and Heessen (1984) manipulated densities of the carabid predator *Pterostichus oblongopunctatus* in field enclosures and found evidence for convergence within two years. Criticisms can be levelled at this technique in that enclosures may prevent emigration. Gould et al. (1990) manipulated densities of gypsy moth by artificially loading eight forest areas with different densities of egg masses to achieve a wide range of infestation levels. This method revealed previously undetected density-dependent mortality in the larval stage, primarily due to two parasitoid species.

Factorial experiments can be used to determine whether factors potentially capable of limiting population numbers combine in a simple additive way or show more complex patterns (synergistic or antagonistic interactions; Chap. 9). A fully factorial experiment is designed to include all possible combinations of two or more factors, and each of the levels within the factors. To be useful, the factorial experiment must be replicated and last long enough to produce time-series data sufficient to assess equilibrium population levels around which numbers fluctuate (Rosenheim, 1998; Sih et al., 1998). Such experiments are used to examine the emergent effects of multiple enemies on prey populations primarily due to intraguild predation (e.g., Mitchell et al., 1992; Costamagna and Landis, 2007; Straub and Snyder, 2008; Frago and Godfray, 2014; Wu et al., 2016; Chailleux et al., 2017; Alhadidi et al., 2019), and how multiple prey species may indirectly impact the role of natural enemies for one another. For example, using field enclosures (Sect. 7.2.3) in an alfalfa field, Cardinale et al. (2003) manipulated the presence of two important

predators, the coccinellid beetle *Harmonia axyridis* and the damsel bug *Nabis* sp., and one parasitoid, *Aphidius ervi*, each individually and in all combinations. They followed the population dynamics of the natural enemies and the prey species, pea aphids *Acyrthosiphon pisum,* and found that the pea aphid was suppressed most in the treatment with all three natural enemies, suggesting a synergistic (greater than additive) effect (Fig. 7.10). However, closer inspection of the data from all treatments and the field revealed that this suppression was mediated by a second prey species, the cowpea aphid *Aphis craccivora*. The cowpea aphid inhibited parasitism of the pea aphid by *A. ervi.* So, when the cowpea aphid was suppressed by the two predators, the parasitoid population increased and suppressed the pea aphid population (Fig. 7.10). These same factorial experimental methods are also used to explore the effects of abiotic factors (Miller et al., 2017) or agricultural manipulations such as mulching (Schmidt et al., 2004), in conjunction with natural enemies.

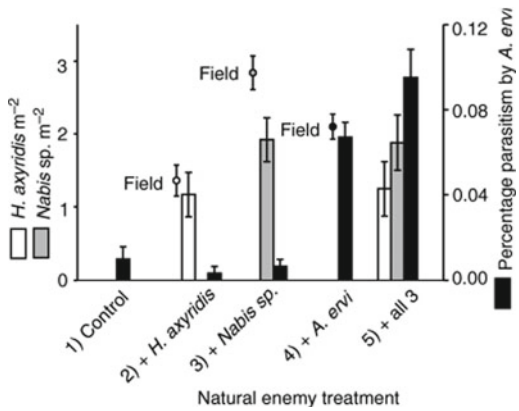

Fig. 7.10 Natural enemies of the pea aphid (*Acyrthosiphon pisum*) treatments on the final date of the study. The densities of predators *Harmonia axyridis* and *Nabis sp.* are based on the final numbers of individuals captured per cage. Parasitism by *Aphidius ervi* is compared among treatments with the ratio [(mummies stem^{-1})/(mummies + *Acyrthosiphon pisum* stem^{-1})]. Bars are the mean ± SE of n = 3 cages for treatments 1–4, and n = 4 cages for treatment 5. For comparison, data points give the naturally occurring densities of enemies in the alfalfa field (from Cardinale et al., 2003, with permission)

7.3.6 Landscape Scale Patterns, and Metapopulation and Metacommunity Dynamics

An insect herbivore usually occurs on a crop or natural plant species that is present at multiple sites in a region, with variable conditions spatially and temporally. In order for a natural enemy to have an impact on the pest, and persist in the long term, it must do so on a landscape scale. The structure of a landscape, such as its configuration, complexity or the fraction of the area cultivated, can determine the regional long-term persistence of an individual natural enemy species, or the composition of the community of natural enemies, and their effectiveness against insect pests. The dynamics of populations in agricultural or other heterogeneous or fragmented landscapes can be modelled as metapopulations. By extension, a community of natural enemies and their prey can be seen as a metacommunity.

In this section we first present aspects of landscape ecology that have been used to make predictions about the impact of landscape structure on biological control. Then we present the existing and potential application of metapopulation and metacommunity ecology for predicting the dynamics of pests and their natural enemies.

Landscape Ecology

Agricultural intensification leads to a simplified landscape. This simplification is associated with decreased biodiversity and increases in economically important pests on cultivated crops. In some cases, the increase in pests is due to decreased effectiveness of natural enemies (Tscharntke et al., 2007; Cohen & Crowder, 2017; Perez-Alvarez et al., 2019). Both conservation and importation biological control may be influenced by the landscape context of the crops. Grab et al. (2018) investigated the relationship between landscape simplification and the importation and conservation biological control of Lygus bugs, *Lygus lineolaris*, in cultivated strawberry. They found increased pest density and reduced parasitism rates of crop pests in landscapes with more intensive agriculture

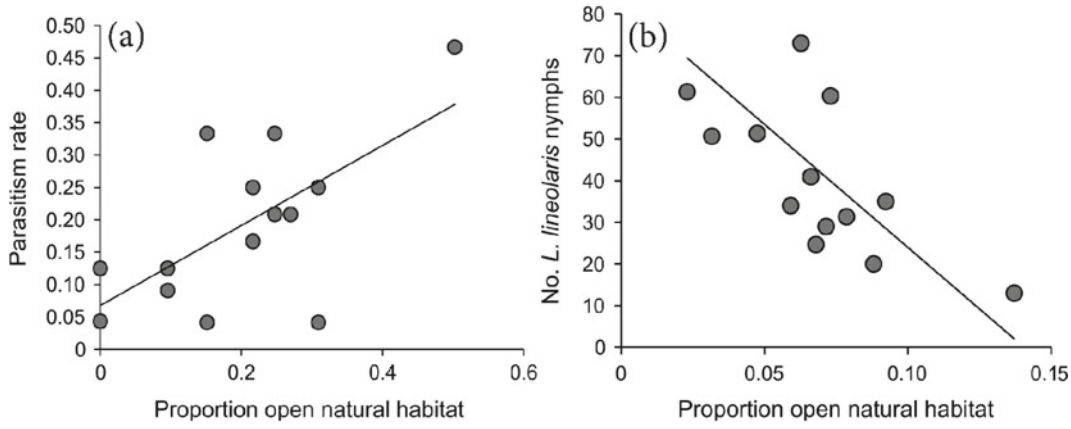

Fig. 7.11 **a** Parasitism rates of *Lygus lineolaris* nymphs by *Peristenus digoneutis* and **b** the average number of *L. lineolaris* nymphs collected from strawberry fields, as a function of the proportion of open semi-natural habitat at 750 m surrounding the sampling location within each strawberry field (from Grab et al., 2018, with permission)

(Fig. 7.11), probably because there were few host and food resources for the parasitoids in simplified landscape (Jonsson et al., 2015). Grab et al. (2018) also found, irrespective of landscape simplification, only introduced parasitoids and no native parasitoids of Lygus bugs in strawberry fields. In this case there was no conservation biological control being provided by parasitoids, even where non-crop habitat was abundant. In contrast, Winqvist et al. (2011) found decreased conservation biological control of aphids on cereal crops by generalist predators in simplified landscapes. However, it is notable that this held for organic farms but not conventional farms (Fig. 7.12), suggesting that landscape at multiple spatial scales is relevant for the maintenance of

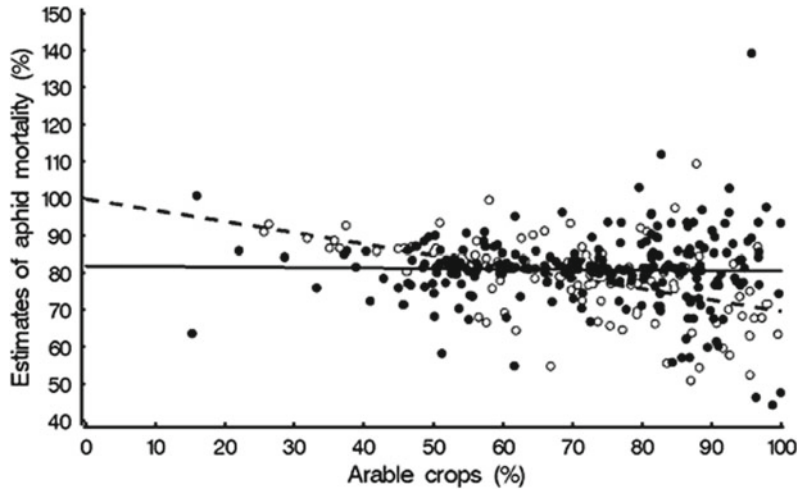

Fig. 7.12 Percentage of aphids eaten (mortality) as a function of percent arable crops in the landscape. The figure was created by plotting residual values for each sampling point (agricultural field) along the landscape gradient. The residual values come from the statistical analysis of variance model, accounting for the variation associated with the random effects of farm nested in region, which leads to apparent aphid mortality of greater than 100% in some fields. Organic fields: open circles and dotted regression line. Conventional fields: filled circles and solid regression line. The interaction is significant, i.e., the significant effect of landscape in the organic fields differs from the insignificant effect in conventional fields (from Winqvist et al. 2011, with permission)

natural enemy populations. These examples illustrate that while biodiversity increases with landscape complexity, increased non-crop habitat in the surrounding landscape does not consistently improve pest management as natural enemy responses can vary from positive to negative (Karp et al., 2018).

Metapopulation and Metacommunity Ecology

The above associations of pests, damage and natural enemies with landscape complexity indicate that the landscape is important for natural enemy populations, and illustrate important ecological patterns, but they do not quantify the dynamics of populations. To do that, we must measure or model change in population sizes over time and/or space. Since most insect pests are distributed in a dynamic patchwork landscape, it is intuitive to place them in a metapopulation context.

A metapopulation is a spatially structured population that persists over time as a set of local populations with limited dispersal between them. Local population processes (reproduction, predation, etc.) occur mostly within the local populations. Between-patch variation in parasitism and predation influence dynamics at the local population level, and dispersal between local populations may account for the persistence of regional populations, despite unstable fluctuations or extinctions at the local level (for reviews see Taylor, 1990, and Hanski, 1998). While the concept existed earlier, the term "metapopulation" was first used by Levins (1969) to describe his model of the potential for insect pest control, including biological control and insecticides, at a regional level. Levins (1969) formulated the rate of change of the fraction of habitat patches occupied by a species in a landscape (p). He used the same logistic differential equation that is used in classical population models (Sect. 7.3.8), but with the number of individuals replaced by the fraction of occupied patches p,

$$\mathrm{d}p/\mathrm{d}t = cp(1-p)ep \qquad (7.5)$$

Here, c is the rate constant for colonisation of empty patches and e is the rate constant for extinction of local populations. Levins' (1969) model includes an intrinsic exponential growth rate cp for colonisation as well as a term that inhibits growth once the metapopulation is large ($-cp^2$), at which point there are few available sites left to colonise. Colonisation is the result of immigration from neighbouring populations. The rate of extinction is proportional to the fraction of occupied patches with the probability of extinction of each patch (e) being independent.

Since Levins' (1969) model, more realistic deterministic models have been developed (Adler & Nuernberger, 1994) as well as probabilistic patch occupancy models, such as the incidence function model that accounts for spatial variation of colonisation and extinction probability (Hanski, 1994), and Bayesian models to address spatial and temporal variability of conditions (Smith et al., 2014), as well as individual agent-based models in which the behaviour of each individual animal is accounted for (Uchmański, 2016).

These models consistently show that persistence at the regional level can be enhanced by dispersal between local populations, provided that: (1) local populations fluctuate asynchronously between habitat patches, (2) predator rates of colonisation are not too rapid relative to those of the prey, and (3) some local density dependence is present. While the degree of density dependence may be quite low, resulting in frequent local extinctions, the metapopulation may persist for a long time (Kean & Barlow, 2000; Hanski et al., 2017). For the most part, these models describe the dynamics of a single predator or parasitoid species reliant on a prey or host resource with independent dynamics. However, they are also applied to systems in which predator–prey dynamics are interdependent (Taylor, 1990; Holt, 1997; Fernandes et al 2022). This includes a number of laboratory studies exploring the effects of spatial structure and dispersal on persistence of predator–prey systems (Huffaker, 1958; Pimentel et al., 1963; Holyoak & Lawler, 1996; Bonsall et al., 2002). Pimentel et al. (1963), for example, examined the interaction between a parasitoid wasp and its fly host in artificial environments consisting of small

boxes connected by tubes. The interaction persisted longer with more boxes and with reduced parasitoid dispersal. Bonsall et al. (2002) developed a similar system of interconnecting boxes to study a bruchid beetle–parasitoid metapopulation interaction, with comparable results. While agreement with theory may be encouraging, the small scale on which these experiments, by necessity, have been carried out, may not reflect processes at the regional metapopulation level.

Metapopulation processes have been detected in some large-scale field predator–prey systems, but joint metapopulation dynamics of the prey with the predator or parasitoid have rarely, if ever, been identified (Walde, 1995; Harrison and Taylor, 1997; Weisser, 2000; Cronin, 2004; Cosentino et al., 2011). One of the best-studied examples involving an arthropod predator–prey system is provided by the Glanville Fritillary butterfly, *Melitaea cinxia*, and its specialist braconid parasitoid, *Cotesia melitaearum*, in the Åland Islands, Finland. In Åland there are around 3,200 suitable dry meadows, of which several hundred are occupied by the butterfly in any one year, and the parasitoid is present in about 10% of the local butterfly populations (Lei & Hanski, 1997; van Nouhuys and Hanski, 2002; Hanski et al., 2017). The dynamics of the butterfly are predicted by metapopulation theory for the observed areas and isolation of habitat patches. The parasitoid also exists as a metapopulation, greatly influenced by the spatial dynamics of the host. However, due to density-dependent hyperparasitism, parasitoid dispersal limitation, and an overarching dependency of the butterfly on host plant quality, the dynamics of the host do not depend on the parasitoid (van Nouhuys & Hanski, 2002; Øpedal et al., 2020). Clearly, more detailed empirical studies of this nature, especially in biological control systems, are required to provide a 'reality check' to the theoretical literature (Cronin & Reeve, 2005).

Insect pests and their natural enemies are part of a community of species in a landscape that is often a patchwork of cultivated and uncultivated land. Thus, the system can be thought of as a metacommunity, which is a community of interacting species made up of local communities linked by dispersal (Leibold et al., 2004). There is a robust literature of metacommunity theory (Logue et al., 2011), but it has not yet been applied quantitatively to questions of efficiency of importation or conservation biological control. Nonetheless, some metacommunity processes are broadly relevant for predicting persistence or dynamics in biological control. One example of this is the consequence of hyperparasitoids for the effectiveness of parasitoids as biological control agents. The patch occupancy model of metacommunity theory (Leibold et al., 2004) predicts that with increasing habitat fragmentation, higher trophic level species such as hyperparasitoids fail to persist because the resources become sparser and more unpredictable at increasing trophic levels (Holt, 2002; Wang et al., 2021). Thus, we predict hyperparasitoids to be present where a plant and insect pest are common in a landscape. This could either stabilise or destabilise effectiveness of biological control (Rosenheim, 1998). A second example of the potential application of metacommunity theory to biological control is the concept of spillover between cultivated and uncultivated parts of the landscape in conservation biological control (Tscharntke et al., 2007; Blitzer et al., 2012). The mass effects model of metacommunity dynamics predicts the movement of individuals within a landscape based on changing resource availability (Leibold et al., 2004). Based on this, a community of natural enemies may persist in a dynamic landscape, such as an agricultural system, in which local populations fluctuate due to changes in resource availability caused by harvest, crop rotation, insecticide application and seasonality.

7.3.7 Analytical Models of Population Dynamics

A continuous-time framework is generally used to model populations with overlapping generations and all-year-round reproduction (Hassell, 2000a, 2000b; Murdoch et al., 2003). In contrast, discrete-time models are more suited for populations with non-overlapping generations that

reproduce in a discrete pulse determined by season. We review simple models of host–parasitoid interactions in discrete-time formalism and describe tools for elucidating their dynamical behaviours. One advantage of simple models is that they are often analytically tractable, i.e., their analysis using mathematical tools can provide generic insights into regulatory mechanisms across parameter regions that lead to stable, unstable, or oscillatory population dynamics. While reviewing classical models introduced decades ago, we also highlight new modelling frameworks and results from recent literature. We emphasise that while we primarily focus on discrete-time models in this chapter, many host–parasitoid systems are more appropriately modelled using the continuous-time framework of Lotka–Volterra predator–prey models (Murdoch et al., 1987, 2003; Ives, 1992; Gurney & Nisbet, 1998; Sanchez et al., 2018; Singh, 2021a).

Simple Discrete-Time Models

Discrete-time models have been a tradition in arthropod host–parasitoid systems; their usage is primarily motivated by the univoltine life-histories of insects residing in the temperate regions of the world. A typical life-cycle consists of adult hosts emerging during spring, laying eggs that hatch into larvae. Hosts then overwinter in the pupal stage and emerge as adults the following year. The host becomes vulnerable to parasitoid attacks at one stage of its life-cycle (typically the larval stage). Adult female parasitoids search and attack hosts during this time window of vulnerability. While adult parasitoids die after this time window, the parasitised hosts support juvenile parasitoids, which pupate, overwinter, and emerge as adult parasitoids the following year. Synchronised life-cycles, with no overlap of generations in both the host and the parasitoid makes discrete-time models highly appropriate for these systems.

A model describing host–parasitoid dynamics in discrete time is given by

$$\begin{aligned} H_{t+1} &= RH_t f(RH_t, P_t) \\ P_{t+1} &= kRH_t[1 - f(RH_t, P_t)] \end{aligned} \tag{7.6}$$

where H_t and P_t are the adult host and the adult parasitoid densities, respectively, at the start of year t, and $R > 1$ denotes the host's reproductive rate. Note that $R > 1$ is needed to avoid population extinction of the host. If the host is vulnerable to the parasitoid at its larval stage, then RH_t is the host larval density exposed to parasitoid attacks. Parasitoids attack host larvae during the vulnerable period leading to two categories of hosts within the population: parasitised and unparasitised larvae. The function $f(RH_t, P_t)$ is the fraction of host larvae escaping parasitism (sometimes referred to as the escape response). In the absence of the parasitoid $f(RH_t, 0) = 1$ and the host population grows unboundedly as

$$H_{t+1} = RH_t.$$

Finally, $RH_t[1 - f(RH_t, P_t)]$ is the net density of parasitised larvae, with each larva giving rise to k adult female parasitoids in the next generation. Renaming variables, where now H_t denotes the host larval density, results in

$$\begin{aligned} H_{t+1} &= RH_t f(H_t, P_t) \\ P_{t+1} &= kH_t[1 - f(H_t, P_t)] \end{aligned}$$

and both forms of the model have been used in the literature.

The simplest formulation of Eq. 7.6 is the classical Nicholson–Bailey model

$$\begin{aligned} H_{t+1} &= RH_t exp(-cTP_t) \\ P_{t+1} &= kRH_t[1 - exp(-cTP_t)] \end{aligned} \tag{7.7}$$

where c represents the rate at which parasitoids locate/parasitise hosts, and T is the duration of the host vulnerable stage (Nicholson & Bailey, 1935). The model assumes that parasitoids search for hosts randomly, are never egg limited, and have rapid handling times. Given the random

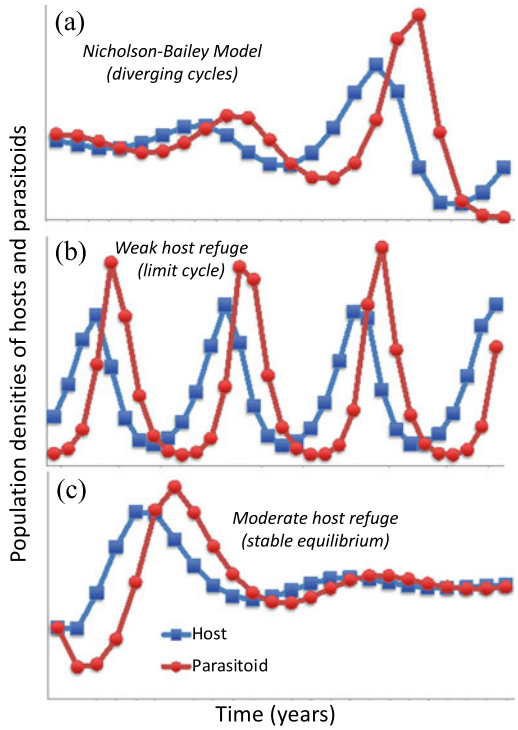

Fig. 7.13 **a** A typical host–parasitoid population time series for the Nicholson–Bailey model (Eq. 7.7), **b** for a model with either a weak host refuge ($\mu = 0.05$ in Eq. 7.12) or c) a moderate host refuge (μ = 0.20 in Eq. 7.12) (Singh & Emerick, 2021, with permission). Host reproductive rate is assumed to be R = 2

host–parasitoid interaction, the number of parasitoid attacks per host follows a Poisson distribution, with mean cTP_t, then the escape response $exp(-cTP_t)$ is the probability of zero attacks in the Poisson distribution. A typical time series of the Nicholson–Bailey model is shown in Fig. 7.13a. Both populations grow at low densities, but at large host densities, the parasitoid begins to overexploit the host. This leads to a crash in the host population, followed by a crash of the parasitoids. These cycles of overexploitation and crashes result in an unstable interaction, with both populations exhibiting diverging oscillations. Before discussing generalisations to the Nicholson–Bailey model, we briefly review mathematical approaches used for dissecting dynamical behaviours.

General Stability Analysis

Given a model of the form represented by Eq. 7.6, one is typically interested in knowing if the model can support a stable host–parasitoid interaction, and if so, then for what parameter values. Simulating the model for a few test parameters (this can be done, for example, in a spreadsheet package, such as Microsoft Excel, by starting with an initial condition and iterating forward in time) can provide the first answers but a systematic quantification of stability regions proceeds along the following steps:

Step 1: Determining the Equilibrium Point

The model's equilibrium or fixed points are the population densities that remain constant across years. Let H^* and P^* denote the host and parasitoid densities at equilibrium, respectively. These equilibrium densities are obtained by first substituting $P_{t+1} = P_t = P^*$ and $H_{t+1} = H_t = H^*$ in Eq. 7.6 and then solving the resulting equations. Note that the model has a trivial equilibrium $P^* = H^* = 0$ where both populations are extinct, but we are primarily interested in the non-trivial equilibrium that represents the coexistence of species. For model 7.6 this non-trivial equilibrium is the solution to the following two equations

$$1 = Rf(RH^*, P^*)$$
$$P^* = k(R-1)H^*$$

Solving these equations for the Nicholson–Bailey (1935) model yields a single non-trivial equilibrium point

$$H^* = \frac{lnR}{(R-1)kcT}$$
$$P^* = \frac{lnR}{cT},$$

where the host equilibrium levels decrease, and the parasitoid equilibrium levels increase, with increasing host growth rate R.

Step 2: Linearising Around the Equilibrium

Real populations are never in equilibrium as environmental fluctuations in model parameters and unmodeled interactions constantly perturb the system out of any equilibrium it may have momentarily reached. If these perturbations transiently decay and the populations return to equilibrium, then the equilibrium point is said to be stable. Alternatively, if perturbations amplify, and the populations increasingly deviate from the equilibrium, then the equilibrium point is said to be unstable. Considering small perturbations $p_t = P_t - P^*$, $h_t = H_t - H^*$ and linearising model nonlinearities in Eq. 7.6 around the equilibrium, results in the following linear discrete-time system

$$\begin{bmatrix} h_{t+1} \\ p_{t+1} \end{bmatrix} = A \begin{bmatrix} h_t \\ p_t \end{bmatrix} \tag{7.8}$$

where A represents a 2×2 matrix whose entries are related to the slope, or sensitivity, of the escape response to population densities (detailed formulas describing each entry of A are provided in the Appendix to Singh & Emerick, 2021). The matrix A is mathematically referred to as the Jacobian matrix and its eigenvalues are intricately linked to the stability of the equilibrium.

Step 3: Checking for Stability

The necessary and sufficient condition for stability of a linear discrete-time dynamical system, such as Eq. 7.8, is that all the eigenvalues of A have an absolute value of less than one (Elaydi, 1996). For a 2×2 matrix, this corresponds to the equilibrium H^*, P^* being stable if the following three conditions all hold

$$\begin{aligned} 1 - Tr(A) + Det(A) &> 0 \\ 1 + Tr(A) + Det(A) &> 0 \\ \\ 1 - Det(A) &> 0 \end{aligned} \tag{7.9}$$

where Tr and Det refer to the trace and the determinant of the matrix, respectively. The equilibrium point is unstable if any one of the inequalities does not hold (we refer the reader to Elaydi, 1996, for details on the mathematical terminology used here).

In many cases these stability conditions can be further simplified. For example, if the escape response $f(P_t)$ only depends on parasitoid density, then the first two conditions always hold, and stability is completely determined by the third inequality $1 - Det(A) > 0$. If $1 - Det(A) = 0$ then the equilibrium point is said to be neutrally stable (on the edge of stability and instability), and both host and parasitoid populations cycle with a period of 6 or higher (Singh & Nisbet, 2007). If $1 - Det(A) < 0$ then the equilibrium is unstable, and populations either show diverging oscillations that grow unboundedly (as in the Nicholson–Bailey, 1935, model; Fig. 7.13a), or they settle into bounded population cycles (i.e., a stable limit cycle; Fig. 7.13b).

Interestingly, for a parasitoid-dependent escape response $f(P_t)$ the inequality $1 - Det(A) > 0$ can be rewritten in a different form

$$\frac{dH^*}{dR} > 0 \tag{7.10}$$

which leads to a simple, yet powerful stability condition: the model's equilibrium is stable, if and only if, the adult host equilibrium density increases with increasing host growth rate R (Singh et al., 2009). Recall that H^* in the Nicholson–Bailey model is decreasing with R, and its instability is reflective of this simplified stability criterion. Using the fact that $P^* = k(R-1)H^*$, the above condition on H^* can also be written in terms of the parasitoid equilibrium density

$$\frac{dP^*}{dR} > \frac{P^*}{R-1}$$

revealing that stability requires parasitoid densities to increase sufficiently rapidly with increasing host growth rate. It is important to emphasise that these simplified conditions are only to be used for a host-independent escape response. When $f(RH_t, P_t)$ depends on both populations,

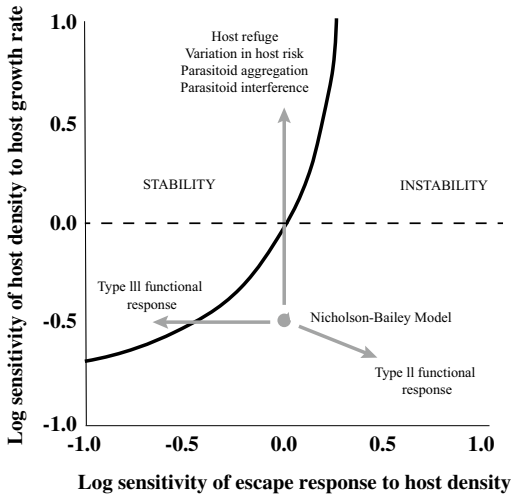

Fig. 7.14 Stability regions for the host–parasitoid model (Eq. 7.6) in terms of the sensitivity of the host density to the host growth rate $\frac{dH^*}{dR}$, and the sensitivity of the escape response to the host density $\frac{df}{dH^*}$ (Singh & Emerick, 2021). The Nicholson–Bailey model corresponds to $\frac{dH^*}{dR} < 0$ and $\frac{df}{dH^*} = 0$ and is unstable. Stability arises in two orthogonal ways: (1) an increase in $\frac{dH^*}{dR}$ to make it positive, which occurs with parasitoid interference and aggregation or host refuge; (2) a decrease in $\frac{df}{dH^*}$ which occurs with a Type III functional response in the parasitoid attack rate (Sect. 1.14). In this figure, the host growth rate is assumed to be $R = 2$ with the axes being dimensionless log sensitivities $\frac{H^*}{f}\frac{df}{dH^*}$ and $\frac{R}{H^*}\frac{dH^*}{dR}$ (modified from Singh & Emerick, 2021, with permission)

the stability conditions (Eq. 7.9) can be graphically represented in terms of two relevant quantities:

$$\frac{dH^*}{dR} \text{ and } \frac{df}{dH^*}$$

where the latter denotes the sensitivity of the escape response to the host density. Figure 7.14 shows that stability region with respect to both these quantities for a general escape response $f(RH_t, P_t)$, and stability is more likely to occur when the escape response is a decreasing function of the host density, rather than an increasing function (Singh & Emerick, 2021).

Expanding the Nicholson–Bailey Model

We next discuss expansions of the Nicholson–Bailey model, emphasising the stabilising and destabilising effects of different mechanisms.

Host and Parasitoid-Dependent Attack Rates.

The Nicholson–Bailey model assumes that parasitoids search and attack hosts with a constant rate c implying a Type I functional response (Sect. 1.14). This assumption is relaxed by considering a Type II functional response (Sect. 1.14). Prior studies have implemented it by modifying the attack rate in Eq. 7.7 to

$$c = \frac{c_1}{1 + c_1 T_h R H_t}$$

where c_1 is the attack rate at low host densities, and $T_h$is the handling time (Rogers, 1972). The net attack rate per parasitoid cRH_t increases with H_t and saturates at $1/T_h$ at high host densities. With this change, the escape response is now an increasing function of H_t and it makes the model even more unstable if we move the Nicholson–Bailey point further to the right, away from the stability boundary in Fig. 7.14. Similarly, a Type III functional response (Sect. 1.14) is incorporated by setting

$$c = \frac{c_1 (RH_t)^q}{1 + c_1 T_h (RH_t)^{q+1}} \tag{7.11}$$

with $q > 0$ capturing the acceleration of attack rate with increasing H_t at low host densities, and analysis shows that such responses fail to stabilise the population dynamics irrespective of the value of q (Hassell & Comins, 1978). The fact that a Type III response is not stabilising in the Nicholson–Bailey framework is surprising, since it is known to have a stabilising effect in the continuous-time framework of Lotka–Volterra models (Murdoch & Oaten, 1975). As discussed below, this discrepancy arises from how the Type III response is phenomenologically

introduced. Indeed, modelling host mortality during the vulnerable period as a continuous-time process confirms the stabilising properties of a Type III response, removing the discrepancy between the two frameworks.

In contrast to a host-dependent attack rate, a parasitoid-dependent attack rate is stabilising. Consider the scenario where the attack rate is proportional to $c \propto P_t^{-m}$ and decreases with increasing P_t due to interference between parasitoids with $0 < m < 1$ quantifying the degree of interference. Since the escape response only depends on P_t, stability can be discerned by simply testing for host equilibrium density increasing with growth rate. Solving for the host equilibrium and applying Eq. 7.10 reveals the system to be stable for

$$\frac{RlnR + 1 - R}{RlnR} < m < 1$$

which corresponds to $0.28 < m < 1$ for $R = 2$, and $0.5 < m < 1$ for $R = 5$ (Hassell & Varley, 1969). In the context of Fig. 7.14, stability arises by moving the Nicholson–Bailey model 'upwards' via a change in the sign of $\frac{dH^*}{dR}$.

Host Refuge.

Some hosts may be in some form of a refuge that protects them from parasitoids. There may be physical refuges in which hosts are protected from parasitism or some hosts may be physiologically immune to parasitoid attack or there may be a seasonal mismatch between host and parasitoid, for instance early developing hosts may avoid searching parasitoids. Refuges may also be statistical: hosts may escape parasitism by chance more often than if parasitoid search was truly random. Host refuges can be incorporated into the Nicholson–Bailey model in two different ways: a constant host density protected from parasitism, or a constant fraction of hosts in the refuge. Both forms of the refuge stabilise host–parasitoid dynamics (Hassell, 2000a, 2000b). For example, a constant host fraction μ in the refuge leads to the following model

$$H_{t+1} = RH_t(\mu + (1 - \mu)exp(-cTP_t))$$
$$P_{t+1} = k(1 - \mu)RH_t[1 - exp(-cTP_t)] \quad (7.12)$$

While a weak refuge results in bounded oscillations, a moderate refuge stabilises the population dynamics (Fig. 7.13b,c). However, stability is again lost for a strong refuge with both hosts and parasitoids growing unboundedly. As before, the stability regime in terms of μ can be obtained by simply checking the criterion given by Eq. (7.10).

Variation in Risk

The stability arising from a host refuge can be generalised under the concept of variation in risk. The Nicholson–Bailey model assumes that all hosts are identical in terms of their vulnerability to parasitism. Perhaps a more realistic scenario is individual hosts differing in their risk of parasitism due to genetic factors, spatial heterogeneities, or the duration or timing of their exposure to parasitism (Bailey et al., 1962). In essence, the product cT in Eq. (7.7) represents the attack rate integrated over time, and by transforming it into a random variable x we obtain

$$H_{t+1} = RH_t \int_{x=0}^{\infty} p(x)exp(-xP_t)dx$$
$$P_{t+1} = kRH_t[1 - \int_{x=0}^{\infty} p(x)exp(-xP_t)dx]$$

where $p(x)$ is the distribution of parasitism risk across hosts (Singh et al., 2009). A key assumption in this formulation is that risk is independent of the local host density if hosts are non-uniformly distributed in space. Assuming $p(x)$ follows a Gamma distribution (a versatile distribution commonly used for capturing skewed population behaviours) with mean $\underline{c}$ and coefficient of variation CV, we obtain the following escape response

$$\int_{x=0}^{\infty} p(x)exp(-xP_t)dx = \frac{1}{\left(1+\underline{c}CV^2P_t\right)^{\frac{1}{CV^2}}},$$

that yields the model

$$H_{t+1} = \frac{RH_t}{\left(1+\underline{c}CV^2P_t\right)^{\frac{1}{CV^2}}}$$

$$P_{t+1} = kRH_t\left(1-\frac{1}{\left(1+\underline{c}CV^2P_t\right)^{\frac{1}{CV^2}}}\right) \quad (7.13)$$

Stability analysis of this model leads to a classical result: $CV > 1$stabilises the population dynamics, irrespective of all other model parameters (R, k, $\underline{c}$) (May, 1978; Chesson and Murdoch, 1986; Murdoch and Stewart-Oaten, 1989; Hassell et al., 1991; Taylor, 1993). The stabilising risk distribution is shown in Fig. 7.15 where most hosts are at low risk, and stability arises from parasitoid attacks being skewed or aggregated towards a small fraction of high-risk individuals, with $1/CV^2$ representing the degree of aggregation. This stability criterion motivated several studies investigating spatial pattern of parasitism in the field, and many data sets were found to be consistent with $CV > 1$ (Pacala & Hassell, 1991).

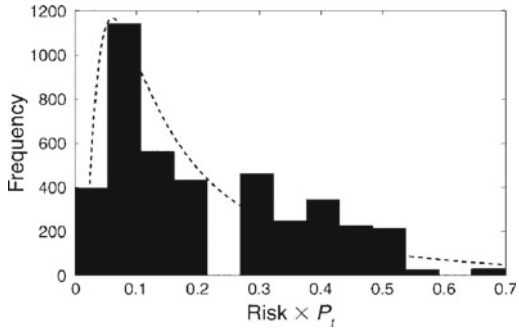

Fig. 7.15 The distribution of risk as obtained from host parasitism data across patches from Reeve et al. (1994) (see Singh et al., 2009, for details on obtaining the distribution of risk). The estimated value of CV for this distribution is 1.31 and the dashed line corresponds to an inverse Gaussian distribution with same mean and CV as the distribution of risk (Singh et al., 2009, with permission)

Recent work in this direction has relaxed the assumption of a Gamma-distributed risk. It turns out that if the host reproduction $R \approx 1$ then $CV > 1$ is the necessary and sufficient condition for stability irrespective of what form $p(x)$ takes (Singh et al., 2009; Singh, 2021b). However, if $R \gg 1$, stability requires a skewed risk distribution with the modal risk being zero (as in the Gamma distribution for $CV > 1$). We illustrate this point with the data presented in Fig. 7.15 where $p(x)$ is approximated by an inverse Gaussian distribution that has a non-zero mode. Despite having a $CV \approx 1.3$, this risk distribution is stabilising only for $1 < R < 2$ (Singh et al., 2009). Interestingly, if the host risk follows an inverse Gaussian distribution and $R > 5$, then the host–parasitoid equilibrium can never be stabilised irrespective of how high CV is. In summary, for host growth rates close to one, sufficient variation in host risk ($CV > 1$) is stabilising. In contrast, at high growth rates, the shape of the distribution for low-risk individuals is crucial in determining stability (Singh et al., 2009).

Semi-Discrete Hybrid Models

As illustrated above, for most host–parasitoid models the escape response is phenomenologically chosen or designed to recapitulate field observations. While these models have tremendously improved our understanding of stabilising processes, mechanistic modelling frameworks are needed to translate insect life-histories and behaviours into discrete-time models. For example, consider a scenario where the parasitoids have a density-dependent mortality from predation or food limitation. It is not obvious how to modify the Nicholson–Bailey model to reflect this density dependence. For this purpose, semi-discrete or hybrid frameworks have been proposed; these use ordinary differential equations to track population densities within the host's vulnerable period during a given year (Rohani et al., 1994; Bonsall and Hassell, 1999; Geritz & Kisdi, 2004; Pachepsky et al., 2008). The solution of the differential equations at the end of the vulnerable period predicts the population densities for the next year. We discuss this semi-discrete formulation in further detail below.

Let τ denote the time within the host vulnerable stage that varies from *0* to *T* corresponding to the start and end of the vulnerable stage. The density of parasitoids (P), unparasitised (L) and parasitised host larvae (I) at time τ within the vulnerable stage of year t, follows the differential equations.

$$\begin{aligned}\frac{dP(\tau,t)}{d\tau} &= -cP(\tau,t)L(\tau,t) - \gamma_P P(\tau,t)\\ \frac{dL(\tau,t)}{d\tau} &= -cP(\tau,t)L(\tau,t) - \gamma_L P(\tau,t)\\ \frac{dI(\tau,t)}{d\tau} &= cP(\tau,t)L(\tau,t) - \gamma_I P(\tau,t). \qquad (7.14)\end{aligned}$$

Here c represents the parasitoid's attack rate, and γ_P, γ_L, γ_I are the mortality rates. Solving the differential equations with initial conditions at the start of the vulnerable period $\tau = 0$

$$L(0,T) = RH_t, P(0,t) = P_t, I(0,t) = 0$$

predicts the parasitised and unparasitised larval population at the end of the season $\tau = T$. This leads to a more general discrete-time model.

$$\begin{aligned}H_{t+1} &= F(H_t, P_t)\\ P_{t+1} &= G(H_t, P_t)\end{aligned}$$

where update functions are obtained by setting $F(H_t, P_t) = L(T,t)$ and $G(H_t, P_t) = kI(T,t)$. As expected, a constant attack rate c with no mortalities ($\gamma_P = \gamma_L = \gamma_I = 0$) results in the Nicholson–Bailey model. Allowing for non-zero density-independent mortalities leads to models very similar in structure to the Nicholson–Bailey models with unstable population dynamics (Singh & Emerick, 2021).

Revisiting Functional Responses

A functional response can be incorporated in the semi-discrete framework by having the attack rate take the form

$$c = \frac{c_1 L(\tau,t)^q}{1 + c_1 T_h L(\tau,t)^{q+1}} \qquad (7.15)$$

where $q = 0$ corresponds to a Type II functional response, and $q > 0$ a sigmoidal Type III response (Sect. 1.14). Singh and Nisbet (2007) show that the resulting discrete-time model based on the hybrid framework is stable for $q > 1$, assuming that the handling time is significantly shorter than the vulnerable stage duration ($T_h \ll T$). With the host density $L(\tau,t)$ decreasing over time due to parasitism, the attack rate (Eq. 7.15) results in a larger fraction of hosts escaping parasitism. This seems to exert a stabilising influence on the population dynamics compared to the phenomenologically chosen attack rate (Eq. 7.11) that is set by the initial host density at the start of season. Thus, systematic consideration of continuous changes in population densities that occur within a season is critical in a discrete-time model formulation. Interestingly, the host equilibrium here is still a decreasing function of the host growth rate (as in the Nicholson–Bailey model), but a Type III functional response induces stability by making the fraction of hosts escaping parasitism a decreasing function of the host density. In the context of Fig. 7.14, this corresponds to shifting the Nicholson–Bailey model to the left in the stability region. Finally, we point out that a Type II response ($q = 0$) is destabilising in both formulations.

Density-Dependent Host Mortality

A key mechanism known to have a stabilising effect on the host–parasitoid populations is the density-dependent self-limitation in the host (May et al., 1981; Neubert and Kot, 1992; Marcinko & Kot, 2020). Density-dependent host mortality can be modelled by assuming that the death rate $\gamma_L = c_h L(\tau,t)$ is proportional to the host density. Solving the continuous dynamics (Eq. 7.14) for a constant parasitoid attack rate yields the following discrete-time model (Singh & Nisbet, 2007).

$$H_{t+1} = L(T,t) = \frac{RH_t exp(-cTP_t)}{1 + c_h RH_t \frac{1-exp(-cTP_t)}{cP_t}}$$

$$P_{t+1} = I(T,t) = \frac{kcP_t}{c_h} ln\left[1 + c_h R H_t \frac{1 - exp(-cTP_t)}{cP_t}\right]. \quad (7.16)$$

In the absence of parasitoids, the host population dynamics follows the Beverton–Holt model

$$H_{t+1} = \frac{RH_t}{1 + c_h TRH_t}$$

Gurney and Nisbet (1998). The discrete-time model (Eq. 7.16) has two non-trivial equilibrium points. The first is the no-parasitoid equilibrium that is set by the strength of the host-density dependence

$$H^* = \frac{R-1}{c_h TR}, P^* = 0$$

and this equilibrium is stable in the overall model (Eq. 7.16) for sufficiently strong density-dependent host mortality c_h verifying

$$lnR < \frac{c_h}{kc}.$$

The second equilibrium, where both host and parasitoid are present, is given by

$$H^* = \frac{exp\left(\frac{c_h}{kc}\right) - 1}{1 - \frac{exp\left(\frac{c_h}{kc}\right)}{R}} \frac{cP^*}{c_h R}, P^* = \frac{lnR - \frac{c_h}{kc}}{cT}$$

and is stable for

$$z^* < \frac{c_h}{kc} < lnR \quad (7.17)$$

where the constant z^* is the solution to

$$z^* + 1 = \frac{R(lnR - z^*)}{R - exp(z^*)}.$$

The different stability regions are summarised in Fig. 7.16, where high values of the ratio c_h/kc (the relative strength of density-dependent host mortality and parasitism) stabilise the no-parasitoid equilibrium, and moderate values stabilise the host–parasitoid interaction. Stability is again lost for low values of $c_h/kc < z^*$ with both populations exhibiting bounded oscillations.

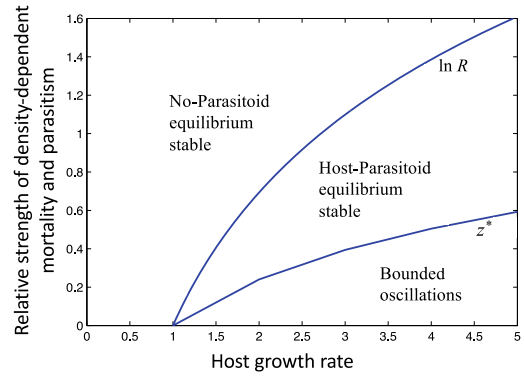

Fig. 7.16 Stability regions for the different equilibriums in the discrete-time model (Eq. 7.16) with respect to c_h/kc (relative strength of density-dependent mortality and parasitism) and host growth rate R. For this plot we assume $k = 1$. From Singh and Nisbet (2007), with permission

The quantity

$$\frac{R\left(exp\left(\frac{c_h}{kc}\right) - 1\right)\left(lnR - \frac{c_h}{kc}\right)}{(R-1)\left(R - exp\left(\frac{c_h}{kc}\right)\right)}$$

is of special interest to biological control, as it represents the ratio of the host equilibrium with parasitoids and without parasitoids. Varying c_h/kc in the stability region (Eq. 7.17) can be used to obtain the maximum level of host depression that is consistent with stable coexistence of both species. This formula predicts an approximate 66% depression of host density for $R = 2$, and 70% depression for $R = 10$ (Singh & Nisbet, 2007). While the parasitoid attack rate is assumed to be constant here, it would be interesting to see how a combination of density dependence in host mortality and other factors, such as parasitoid handling times, egg limitation, host-to-host differences in parasitism risk, shape stability and the degree of host suppression.

Density-Dependent Parasitoid Mortality

Singh and Nisbet (2007) show that density dependence in the parasitoid mortality rate modelled by taking $\gamma_P = c_P P(\tau, t)$ in Eq. 7.14 results in a model identical to the one obtained by assuming a Gamma-distributed host risk. The

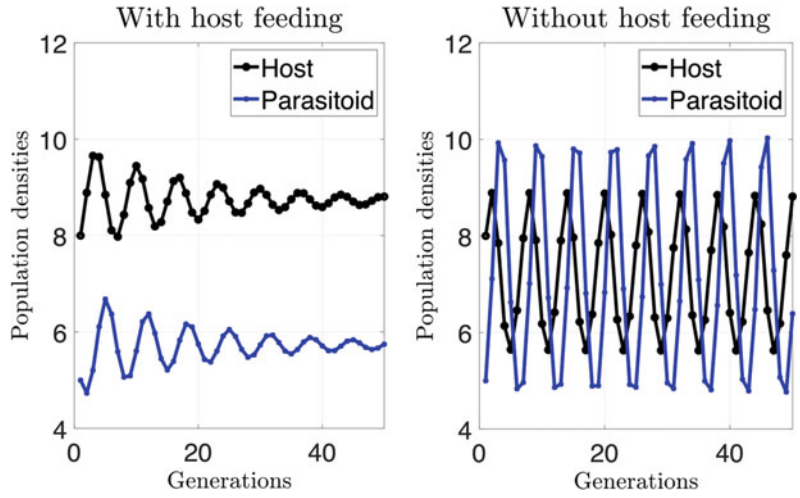

Fig. 7.17 Coupling host feeding with a quadratic parasitoid attack rate stabilises otherwise neutrally stable host–parasitoid population dynamics (from Emerick and Singh, 2016, with permission)

only difference being that $\underline{c}$ in Eq. 7.13 is replaced by c, and CV^2 by $\frac{c_P}{c}$. As discussed earlier, stability arises in the model when $CV > 1$, implying that strong density-dependent parasitoid mortality $c_P > c$ can stabilise the host–parasitoid interaction.

Host Feeding

Recent work has used the semi-discrete framework to investigate the effect of host feeding, which refers to the tendency observed in a number of species of parasitoids for adult females to use some host individuals as a food source rather than an oviposition site. Emerick and Singh (2016) considered each adult female parasitoid to be in one of two states: without eggs or with just one egg to lay. Host feeding results in the death of the host with an eggless parasitoid gaining resources to produce an egg, whereas host parasitism by an egg-carrying parasitoid results in a parasitised host and an eggless parasitoid. Analysis of such simple models using the semi-discrete framework shows that while host feeding by itself cannot stabilise the otherwise unstable Nicholson–Bailey model, it can have a stabilising effect when coupled to other stabilising mechanisms. These results complement several studies incorporating the effects of host feeding in continuous-time models, where host feeding can have stabilising effects (Yamamura & Yano, 1988; Kidd & Jervis, 1991; Murdoch et al., 1992; Briggs et al., 1995), but a delay in egg production following host feeding is destabilising (Shea et al., 1996). This point is illustrated by considering a quadratic Type III functional response $c = c_1 L(\tau, t)$ with the parasitoid attack rate (for both host feeding and parasitism) increasing linearly with the larval population density. It is important to point out that such a functional response leads to a neutrally stable host–parasitoid equilibrium in the absence of host feeding in the semi-discrete formalism (Singh & Nisbet, 2007). Interestingly, incorporation of host feeding in this model exhibits a stable host–parasitoid equilibrium for all values of R. Thus, the inclusion of host feeding converts a neutrally stable equilibrium to a stable one (Fig. 7.17).

Modelling Host–Parasitoid Communities

Most parasitoid species attack more than one species of host, and most host species are attacked by several species of parasitoids. Having presented insights from two-species host–parasitoid models, we now discuss progress towards expanding these models to more than two species. The simplest case is a three-species community, either consisting of one host species and two parasitoid species, or two host species attacked by a common parasitoid.

Two Host Species Attacked by a Common Parasitoid

We first consider the scenario where two different host species are attacked by the same parasitoid species. In this case, the overall population dynamics can be described by the model.

$$H_{t+1} = R_H H_t f_H(P_t, H_t, G_t)$$

$$G_{t+1} = R_G G_t f_G(P_t, H_t, G_t)$$

$$P_{t+1} = k_H R_H H_t[1 - f_H(P_t, H_t, G_t)] + k_G R_G G_t[1 - f_G(P_t, H_t, G_t)]$$

where H_t, G_t are the population densities of the two host species with reproduction rates R_H, R_G, escape responses f_H, f_G, and the number of parasitoids emerging from each parasitized host k_H, k_G, respectively. This indirect interaction between two hosts (due to a shared parasitoid) has been referred to as apparent competition (Holt & Lawton, 1993; McPeek, 2019). If the escape responses f_H, f_G only depend on the parasitoid density P_t, then coexistence of both hosts is not possible, and the host with the lower reproduction rate is driven to extinction. These observations have been replicated in laboratory experiments with two moth species, *Plodia interpunctella* and *Ephestia kuehniella,* that were shared by a common parasitoid, *Venturia canescens*. *P. interpunctella* and *E. kuehniella* were separated and thus could not compete with each other for resources but the shared parasitoid could access both species: either of the two hosts could persist with the parasitoid in a two-species interaction but in the three-species interaction the *Ephestia* population became extinct (Bonsall & Hassell, 1998). Thus, some form of host-density dependence in one or both of the escape responses is necessary for the stable coexistence of all three species.

Recall from Eq. (7.13) that aggregation of parasitoid attacks on a small fraction of high-risk hosts can stabilise the population dynamics. Analysis of these models in the context of apparent competition shows that while such aggregated attacks always end up excluding one of the host species, combining it with some form of host-switching by the parasitoid leads to persistence of all the three species (Bonsall & Hassell, 1999). Along the same theme, recent work has considered a Type III functional response towards just one host species. More specifically, the parasitoid attacks host G with a constant rate (as in the Nicholson–Bailey model) but attacks host H with an accelerating rate analogous to Eq. (7.15) with exponent q. Discrete-time models formulated using the hybrid framework reveal that a Type III functional response towards just one species is sufficient to stabilise the population dynamics of apparent competition, even though the interaction between the parasitoid and host G by itself is unstable (Singh, 2021c). Hence, removal of H from a stable three-species interaction will destabilise the resulting two-species interaction. For example, when $R_H = R_G = 2$ a strong acceleration of parasitoid attack rate towards H with $q > 1.15$ is sufficient for stable coexistence of both hosts. Note that this value of q is higher than that needed to stabilise the parasitoid interaction with just H, in which case $q > 1$ is required.

Two Parasitoid Species Sharing a Common Host

A complementary scenario to that presented above is when a single host species is being used as a resource by two different parasitoid species. Here the model takes the form

$$H_{t+1} = RH_t f_P(P_t) f_Q(Q_t)$$

$$P_{t+1} = k_H RH_t[1 - f_P(P_t)]$$

$$Q_{t+1} = k_Q RH_t f_P(P_t)[1 - f_Q(Q_t)]$$

where now P_t and Q_t are the population densities of the two parasitoid species and f_P, f_Q are the respective parasitoid-dependent escape responses. The implicit assumption here is that the parasitoids attack different developmental stages of the host: P attacks first and the host density escaping parasitism $RH_t f_P(P_t)$ is then exposed to attacks from Q. These models have been investigated in the context of aggregated parasitoid attacks, with f_H and f_Q taking a form like the

escape response in Eq. (7.13). In this case, coexistence of parasitoids is possible when the degree of aggregation (i.e., CV values in Eq. 7.13) for both consumers is greater than that needed for the stability of a single parasitoid–single host interaction (May & Hassell, 1981; Kakehashi et al., 1984). It is interesting that coexistence is also possible when only one of the parasitoids exhibits aggregated attacks, and the other attacks randomly with a constant rate, but the region of parameter space permitting coexistence is significantly reduced.

Recent work has introduced a general class of models to explore multi-parasitoid dynamics

$$H_{t+1} = RH_t f(P_t, Q_t)$$
$$P_{t+1} = k_H RH_t[1 - f(P_t, Q_t)]g(P_t, Q_t)$$
$$Q_{t+1} = k_Q RH_t[1 - f(P_t, Q_t)][1 - g(P_t, Q_t)]$$

where the escape response $f(P_t, Q_t)$ is the fraction of hosts escaping parasitism from both parasitoids, and $0 \leq g(P_t, Q_t) \leq 1$ is the competition response representing the fraction of parasitised larvae that will develop into adult parasitoids P_{t+1} in the next generation. Similarly, $1\text{-}g(P_t, Q_t)$ is the fraction of parasitised larvae that will develop into adult parasitoids Q_{t+1}. To be ecologically relevant, $g(P_t, Q_t)$ is an increasing function of P_t (i.e., with increasing density P_t a larger pool of the parasitised larvae belongs to parasitoid P). Analysis of this general model reveals that the stable coexistence of both parasitoids depends on two remarkably simple criteria. The first criterion is the same as that for a single parasitoid–single host interaction: the adult host equilibrium density should increase with R, as in Eq. (7.10). The second criterion is that any increase in P_t density should not cause a large increase in $g(P_t, Q_t)$. For a symmetric interaction $k_H = k_Q$ where both parasitoids have similar equilibrium densities, the second criterion reduces to

$$\frac{P^*}{g(P^*, Q^*)} \frac{\partial g(P_t, Q_t)}{\partial P_t}\Big|_{P_t = P^*, Q_t = Q^*} < \frac{1}{2}$$

implying that the dimensionless log sensitivity of the competition response with respect to P_t must be less than half (Singh & Emerick, 2022). It will be interesting to expand these results to more complex communities with specialist parasitoids attacking their hosts, and generalist parasitoids sharing hosts creating both direct competition between consumers, and apparent competition between host species. However, this analytical modelling approach is likely to rapidly reach the limits of mathematical tractability as the size of the considered communities expands.

7.3.8 Confronting Models with Field Data

Introduction

The value of analytical models is to provide a framework for exploring how processes such as density dependence, and life-history traits such as search efficiency, can influence the dynamics of consumer–resource interactions. Such models are based on a minimal set of biological details but have the advantage that they are simple enough to allow analytical solutions to be found for equilibrium densities and stability properties (Sect. 7.3.7). While predictions from modified analytical models have been compared to observed changes in host and parasitoid abundance under field conditions (Hassell, 1980), in general, such models are too simplistic to capture the greater complexity associated with the dynamics of particular species under field conditions (May & Hassell, 1988). Consequently, there have been two approaches used to confront models with field data on the density of host and natural enemy populations over time: (1) comparison of simulation model predictions and observed data, and (2) time-series analyses for selecting a statistical model that best describes, or fits, the observed data. For both approaches the time frame of the observed data can vary from a single growing season for a multivoltine population in an agricultural crop to multiple years for a univoltine population in a forest stand.

Comparing Simulation Model Predictions to Observed Data

This approach requires the construction of a simulation model that can be parameterised from independent laboratory or field data and validated by graphical comparison of model predictions to observed data representing a time series of population densities. Simulation models generally include additional biological details such as temperature-driven development rates, other factors that have an important influence on either reproductive or mortality rates of the host population, and a broader set of life-history traits of the natural enemies. In addition, simulation models can differ in number of trophic levels represented, and from intermediate to extreme complexity, depending on the level of knowledge of a particular system. Although complex simulation models with extensive biological detail were developed early on in response to the availability of increased computing power, models of intermediate complexity are now favoured as they have the distinct advantage of greater clarity and generality (Godfray & Waage, 1991). Following graphical comparison of predictions and observations, it is good practice to test the robustness of a simulation model using a sensitivity analysis in which parameter values in the model are adjusted by a small amount (often ± 10%). Three examples of simulation models that evaluate the effect of parasitism on agricultural pests are considered below, representing increasing levels of complexity.

Barlow and Goldson (1993) developed a two trophic level simulation model of intermediate complexity for the lucerne weevil *Sitona discoideus*, a univoltine pasture pest in New Zealand, to evaluate the extent to which parasitism of adult weevils by an introduced multivoltine parasitoid *Microctonus aethiopoides* contributed to a 75% reduction in reproductive weevil densities in the autumn. A discrete-time simulation model for peak autumn weevil densities included variables for population growth to a larval carrying capacity and effects of parasitism, drought and larval competition. The parasitoid has four to six generations each year, but mortality from parasitism in two of these generations was identified as being of greatest importance. One generation caused mortality immediately after adult weevils return to pastures after summer aestivation, and a second caused extensive mortality among reproductive adults in the autumn. Both parasitoid generations were included in the model and parasitism was based on an analytical model for parasitoid interference (Hassell & Varley, 1969). One of the strengths of this study was that all the variables in the model could be parameterised exclusively from independent field data without the need for detailed laboratory experiments. When validated against observed data collected over a 16-year period in Darfield, New Zealand (Kean & Barlow, 2000), the model provided a close match to the field data for adult weevil densities, but slightly overestimated the extent of parasitism (Fig. 7.18).

Murdoch et al. (2005) conducted a unique field experiment which demonstrated that the abundance of California red scale, *Aonidiella aurantia*, on lemon trees in coastal California, USA, is controlled by its introduced parasitoid *Aphytis melinus* and that the interaction is locally stable on individual trees. The experiment compared scale and parasitoid populations over a period of 18 months on both caged and uncaged trees. For the caged trees, additional scale crawlers were introduced over the first three months to elevate scale abundance to levels observed during outbreaks. The results were then compared to the predictions from a simulation model. This was an extension of an analytical stage-structured parasitoid–host model by Murdoch et al. (1992) that was updated to include temperature-driven maturation of both scale and parasitoid life stages on a daily basis. Many of the known biological details of the interaction, which had been studied over a period of 20 years, were included in the model, such as the effects of host feeding, sex allocation and a type I functional response with egg limitation for the parasitoid and an invulnerable adult stage for the scale. All variables in the model were parameterised independently from both laboratory and field data. The outcome of the experiment was that parasitism by *A. melinus* reduced scale

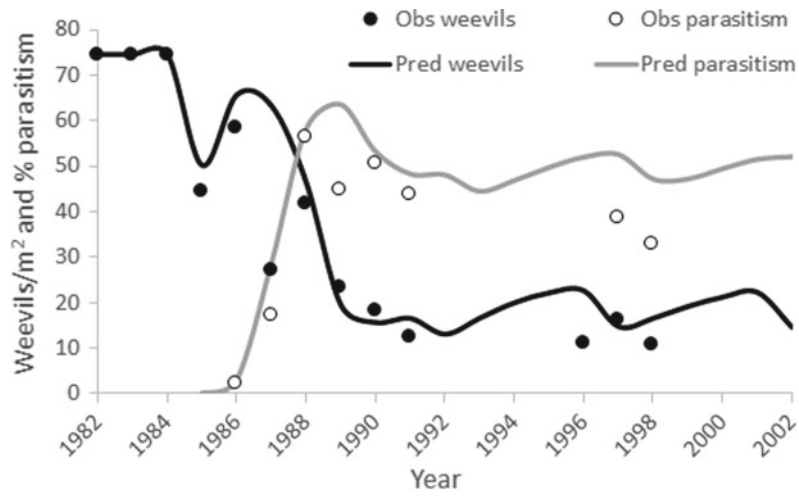

Fig. 7.18 Observed (circles) and model-predicted (lines) peak alfalfa weevil densities in the autumn (black line and solid circles) and levels of parasitism by *Microctonus aethiopoides* (grey line and open circles) in pastures in New Zealand (modified from Kean & Barlow, 2000, with permission)

densities on caged trees to the level present on open control trees within two months of the termination of scale crawler releases, and that thereafter both scale and parasitoid densities were identical on caged and open trees and showed very little variation in abundance over time. The model almost exactly predicted the pattern of change in scale and parasitoid densities in the closed cages, and the prediction proved surprisingly robust to a sensitivity analysis of each set of variables. The close match between model prediction and experimental data provided strong evidence that the model successfully captured the mechanisms by which *A. melinus* was able to both suppress and stabilise the scale population on individual trees.

Gutierrez and colleagues developed a tritrophic approach to simulation modelling in which a generalised supply–demand function is used for resource acquisition and conversion for all trophic levels from plant to predator or parasitoid (Freckleton & Gutierrez, 1996). The supply–demand function is an example of a ratio-dependent Type II functional response for resource acquisition that is driven by per unit biomass demand for resources to support growth and reproduction coupled with a numerical response for resource conversion that is based on assimilation and respiration (Gutierrez et al., 1994). The simulation model can include age structure and temperature-driven functions for maturation, reproduction, and resource acquisition and conversion, as needed for application to a specific system. This approach was used to explore the seasonal dynamics of the very successful biological control of cassava mealybug, *Phenaccocus manihoti*, by an introduced parasitoid *Anagyrus lopezi* and native coccinellids in Africa (Gutierrez et al., 1993). The model was parameterised using a combination of independent laboratory and field data and model fitting for those parameters that could not be determined independently. The model accurately predicted the growth of different components of the cassava plants over the growing season in Ibadan, Nigeria in 1983–1984 and mostly captured the seasonal fluctuations in abundance of mealybugs in the presence of the introduced parasitoid and native predators. The importance of predation and parasitism was evaluated by adding or deleting each component one at a time in a series of simulations, which showed that native coccinellids contributed little to control of the mealybug. The presence of the parasitoid could predict the extent and pattern of mealybug suppression, but only when a constant low level of parasitoid immigration was included. This suggested that the parasitoid may not be able to persist at a local scale following periods of low mealybug densities, but that it has the capacity to build populations sufficiently quickly to suppress mealybug abundance upon recolonisation.

A similar approach has been used to compare parasitoid species for the biological control of the coffee bean borer (Rodríguez et al., 2017; Cure et al., 2020; Sect. 7.4.3).

Time-Series Analysis Using Statistical Models

The time-series analysis approach to confronting statistical models with field data requires the use of longer-term data sets that typically span many generations of an herbivore and its predators and/or parasitoids. In contrast to simulation modelling, time-series analysis involves the fitting of linear, nonlinear or autoregressive statistical models to data using either a least-squares (for normal error distributions) or maximum likelihood (for non-normal error distributions) procedure. Separate models are developed for host and natural enemy populations, and the dependent variable can either be the natural log (ln) of population density at time $t + 1$ or the *per capita* rate of population change in ln population density from time t to time $t + 1$. The independent explanatory variables in a time-series model often include effects of host-density dependence (population density in previous generations), and effects of parasitism rate (proportion parasitised), predation rate (proportion predated), food quality and climatic factors. The goal is to find the simplest model that best fits the observed data, and to make sure that the fitted coefficients for the independent variables make good biological sense.

At its simplest, Münster-Swendsen and Berryman (2005) used multiple regression analysis (Chap. 9) (and the coefficient of determination as a measure of goodness of fit) to determine the best statistical model to describe the cyclic dynamics of the spruce needleminer *Epinotia tedella* in Denmark over a 19-year period based on host-density dependence and parasitism by two parasitoid species. The best-fit model was a logistic model for the *per capita* rate of population change ($R_t = \ln[N_t] - \ln[N_{t-1}]$):

$$R_t = a + bN_{t-1} + c\left(\frac{A_{t-1} + B_{t-1}}{N_{t-1}}\right) \qquad (7.18)$$

where N is the density of needleminers, A the density of *Apanteles tedellae*, B the density of *Pimplopterus dubius*, and a, b and c are fitted constants. In this logistic model the effect of parasitism was represented by the ratio of parasitoids to hosts (Berryman et al., 1995) and accounted for 73% of the variation in R_t.

The analysis of time-series data is often based on autoregressive models to account not only for time lags in the effect of the independent variables (typically limited to no more than three generations to facilitate interpretation; Royama, 1992), but also for the effects of moving averages or temporal trends in the data. Time-series models are fitted to data using maximum likelihood, and the Akaike Information Criterion (AIC), which allows for maximisation of descriptive power and minimisation of the number of variables fitted, is used to select between competing models (Chap. 9). This approach has been applied to the analysis of changes in population densities of the yew gall midge *Taxomyia taxi* and its two parasitoids *Mesopolobus diffinis* and *Torymus nigritarsus* from 1967 to 2001 in the UK (Redfern & Hunter, 2005). The gall midge has a complex life-cycle which takes either one (bud galls) or two (shoot galls) years to complete and has distinct two-year generations in odd and even years. In contrast, *M. diffinis* has three generations per year and *T. nigritarsus* has a single generation each year. Separate models were fitted to log densities for the one-year and two-year life-cycles of the gall midge and to both parasitoids, and the independent variables included host-density dependence, parasitism (log density of *T. nigritarsus* and *M. diffinis*), tree vigour (width of growth rings) and climate (mean monthly precipitation, mean monthly maximum temperature and mean monthly minimum temperature). For simplicity, we consider only the best-fit models for the log densities of the two-year gall midge life-cycle (T) and its main parasitoid *T. nigritarsus* (N)

$$T_t = 0.89T_{t-2} - 0.33N_{t-2} + 0.02PREC_t - 0.57$$

$$N_t = 0.86N_{t-1} + 0.23N_{t-2} - 0.48N_{t-3} + 0.005T_{t-2} + 0.56 \quad (7.19)$$

where *PREC* denotes mean monthly precipitation and the subscript t denotes year. Over the 35-year period, gall midge densities were positively influenced by densities in the previous generation (t-2) and precipitation in the current year (t), and negatively influenced by *T. nigritarsus* densities in the previous generation. For the parasitoid, densities were positively influenced by gall midge densities in the previous generation, with a more complex effect of density dependence that had a positive influence for one-year and two-year time lags, but a negative influence for a three-year time lag. Further analysis from a simulation of the four time-series models (Fig. 7.19) showed (1) that *T. nigritarsus* drives cycles in the abundance of the yew gall midge, with an approximate 14-year periodicity, and (2) that stochasticity in climate variables is needed to prevent the cycles from having a constant periodicity.

While time-series analysis allows for testing of hypotheses about the role of predation or parasitism in driving the dynamics of prey or host populations, standard autoregressive models do have some limitations. For example, they do not incorporate more detailed functions for parasitism and/or predation, nor do they take the effects of measurement error in the estimation of densities from field populations or of environmental stochasticity on population processes into consideration. Although approaches have been developed to include both functional expressions of parasitism and other sources of variability into autoregressive models, complex mathematical methods are required both for model fitting and model selection. Probably as a consequence, these approaches have had limited application to date (Turchin, 2003; Kendall et al., 2005).

An alternative approach to addressing the problem of fitting statistical models of predator–prey interactions to time-series data that include observation error and process noise is through state-space or hierarchical models (de Valpine, 2003). Although state-space models are also more complex, recent developments have introduced maximum likelihood methods for parameter estimation (Chap. 9), model selection using AIC (Chap. 9), and one-step-ahead predictions to summarise model fit. This approach has been used to determine the extent to which the interaction between the woolly bear caterpillar, *Platyprepia virginalis*, and its tachinid parasitoid *Thelaira americana* (both univoltine) affects the

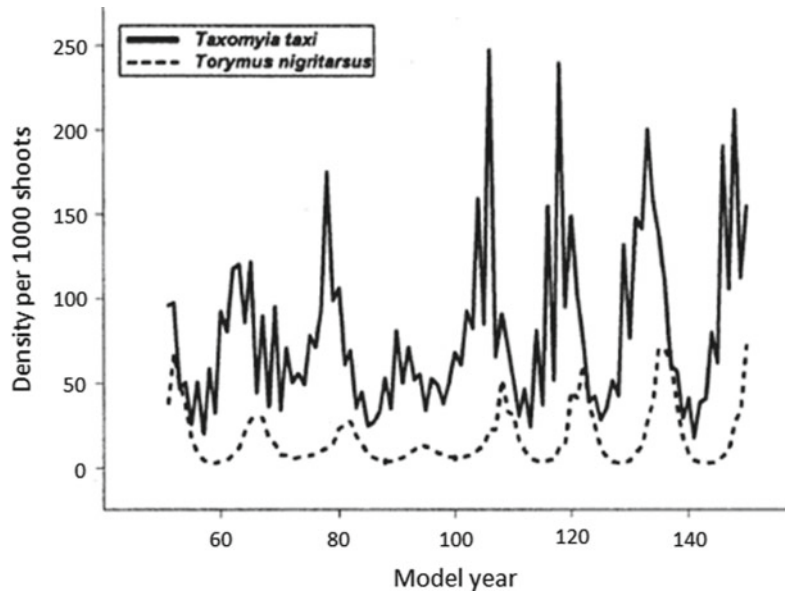

Fig. 7.19 Stochastic simulation of the coupled interaction between the 2-year life-cycle of the yew gall midge (solid line) and its main parasitoid *Torymus nigritarsus* (dashed line) based on time-series models fitted to observed data (from Redfern & Hunter, 2005, with permission)

dynamics of both species from a 21-year time series of observational data from California, USA (Karban & de Valpine, 2010). The state-space models for each species included environmental stochasticity, measurement error for both caterpillar densities and percent parasitism, Ricker and Gompertz functions for density dependence, a Nicholson–Bailey function for parasitism, and precipitation. Despite parasitism of up to 70%, both caterpillar and tachinid densities were shown to be driven by a combination of density dependence and precipitation rather than by the parasitoid–host interaction, although there was marginal evidence that tachinid abundance was driven by caterpillar abundance. State-space models have also been developed to analyse time-series data on the within-seasonal dynamics of pea aphid, *Acyrthosiphon pisum*, in alfalfa fields in Wisconsin, USA (Gross et al., 2005), and of cotton aphid *Aphis gossypii* in cotton fields in California, USA (de Valpine & Rosenheim, 2008). Although both studies found evidence for density dependence in the aphid populations, neither found a role for parasitism or predation in determining the pattern of seasonal dynamics. Nonetheless, state-space models may well offer new opportunities for the analysis of time-series data in the future and for a more rigorous approach to confronting models with field data.

7.4 Practice of Importation Biological Control

7.4.1 Overview

Importation biological control, also known as classical biological control, is the control of invasive pests through the deliberate introduction of specialist natural enemies from the geographic region of origin of the pest (Heimpel & Mills, 2017). Having begun as a very pragmatic and empirical approach to pest management in the late 1800s, the practice of biological control generated tremendous interest among population ecologists who sought to place it in a rigorous scientific framework that could both explain the successes achieved and provide additional guidance for the future (McEvoy, 2018). While theoretical ecology has made some important contributions to our understanding of consumer–resource interactions, our ability to predict success and to select natural enemy species with traits that are most likely to lead to success remains an elusive goal (Heimpel & Mills, 2017; Segoli et al. 2023).

The practice of importation biological control involves a complex sequence of steps that must be carefully followed to maximise the chances for success (Van Driesche & Hoddle, 2000). These steps include characterisation of the pest, foreign exploration for specialised natural enemies, selection and screening of candidate control agents, field release of approved control agents, and monitoring for establishment, spread and programme evaluation (Fig. 7.20). In this section we will explore historical patterns of success, criteria for selecting natural enemies, non-target effects, and methods for natural enemy release and programme evaluation.

7.4.2 Historical Patterns of Success

One of the earliest and perhaps the best-known examples of biological control is that of the cottony cushion scale, *Icerya purchasi*, as an invasive pest of citrus in California, USA (Caltagirone & Doutt, 1989). In this example, the vedalia beetle, *Rodolia cardinalis*, was imported from Australia in 1888 as a specialist predator and deliberately released in citrus groves where it brought about complete suppression of the pest. The degree of success of the programme was both compelling and inspiring, although the causal nature of the impact of the vedalia beetle in suppressing scale populations to very low levels of abundance was verified only more recently through unintended insecticide disruption (Grafton-Cardwell, 2015).

Cock et al. (2016) reviewed a database (BIOCAT2010) of introductions of insect biological control agents for the control of insect pests to the end of 2010. The historical record shows 6,158 introductions, using 2,384 different biological control agents against 588 pest species

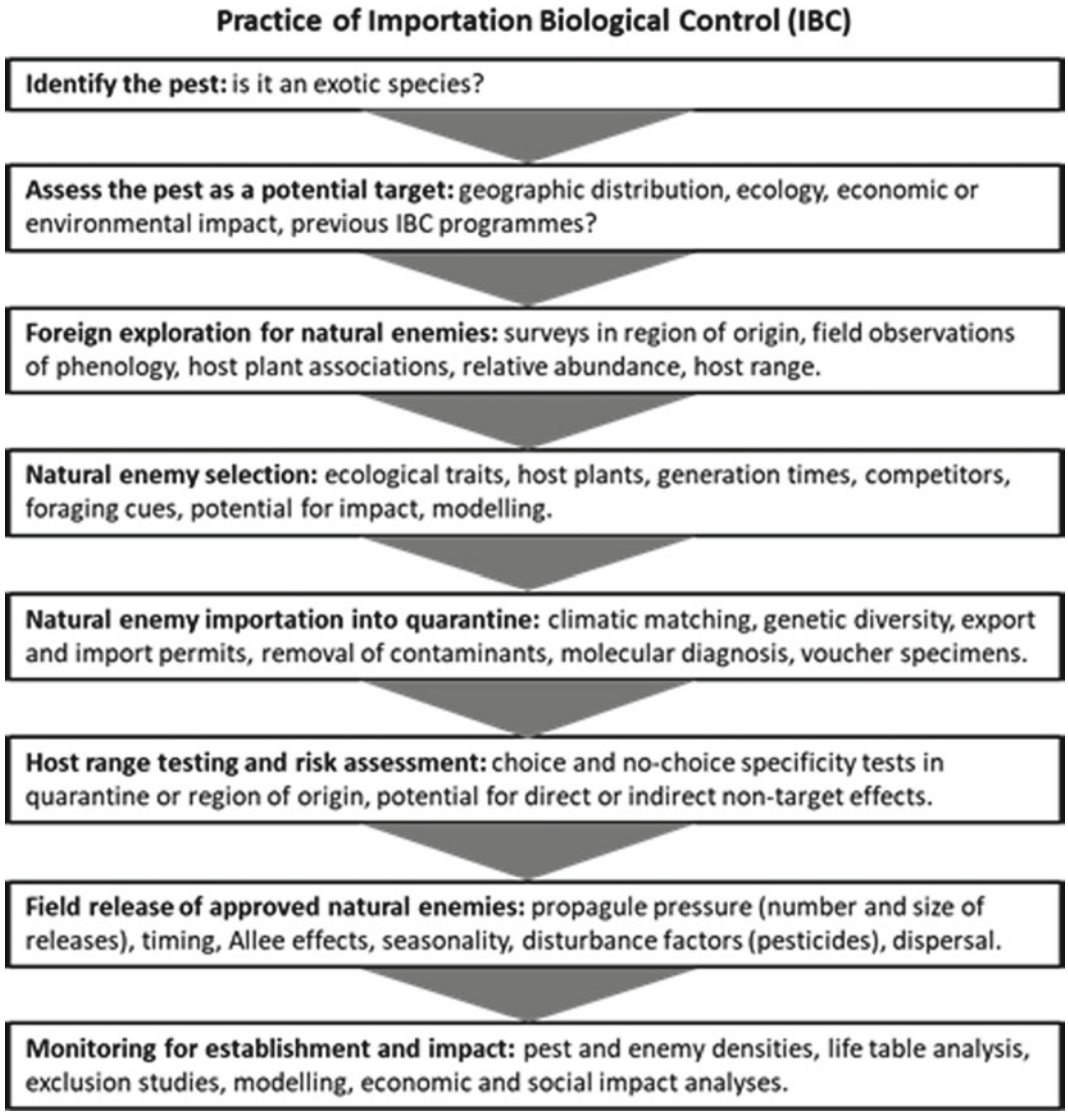

Fig. 7.20 The sequence of steps in an importation biological control programme (modified from Thacker, 2002, with permission)

in 148 countries. In analysing the patterns of success, three important trends were apparent. Firstly, the number of natural enemy introductions per decade increased from the 1870s through to the 1970s, with temporary declines during the two world wars, but subsequently decreased dramatically each decade from the 1970s to the 2000s (Fig. 7.21a). Secondly, over the same period of time, the number of countries with successful introductions each decade has shown a steady increase through to the 1990s. These patterns reflect (1) the increase in unintentional introductions of exotic species via global networks of trade and transport over time (Banks et al., 2015), (2) an increased concern since the 1970s regarding the safety of biological control that has constrained the number of introductions per decade since then, and (3) an increase in both level of interest and effort devoted to biological control as an approach to

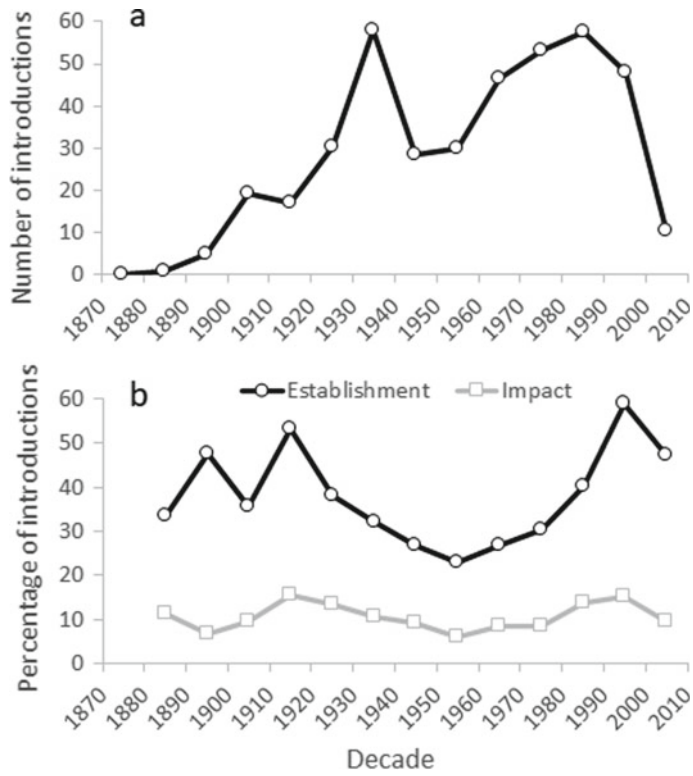

Fig. 7.21 Trends in the historical record of introductions of biological control agents, indicating, **a** the pattern in number of introductions by decade, and **b** the pattern in percentage of introductions that established (open circles, black line) and provided successful control (open squares, grey line) (modified from Cock et al., 2016)

pest management (Cock et al., 2016). Of the total introductions, 2,007 (32.6%) led to establishment, and 620 (10.1%) resulted in satisfactory control being reported against 172 (29.3%) different pest species (Fig. 7.21b). There is also some evidence that the efficiency of establishment and success have improved since the 1950s, but the improvement is moderate, from 20 to 40% for establishment and from 8 to 15% for success. Nonetheless, these positive trends reflect the greater research effort now made to optimise the chances of success and the increased confidence in importation biological control as a viable pest management strategy against a backdrop of the risk-averse culture that has developed in some key countries in recent years (Heimpel & Cock, 2018).

The historical record of importation biological control indicates that not all groups of insect pests have attracted the same level of attention or success. The Hemiptera (Homoptera), which includes scale insects, mealybugs, aphids and whiteflies, and Lepidoptera (moths and butterflies) are the two groups of insect pests that have attracted the greatest number of introductions (Waage & Mills, 1992). It is also notable that success has been consistently greater for homopteran than for lepidopteran pests (Mills, 2006a). Introductions for the control of beetle pests, such as Curculionidae and Chrysomelidae, although less well represented, have also shown high rates of establishment and success (Heimpel & Mills, 2017).

Biological control agents introduced for the control of arthropod pests include, in order of frequency of use, insect parasitoids, arthropod predators, microbial pathogens (fungi, viruses, microsporidia, bacteria and oomycetes) and insect parasitic nematodes (Hajek & Eilenberg, 2018). Among the insect parasitoids, tachinid flies and ichneumonid wasps have shown lower rates of establishment, while aphelinid, encyrtid, eulophid and scelionid wasps have had the highest rates of success, and trichogrammatid and pteromalid wasps have shown the lowest rates of success (Heimpel & Mills, 2017). In

general, arthropod predators have been less successful in importation biological control than insect parasitoids (Kimberling, 2004; Heimpel & Mills, 2017). Rates of establishment have been low in most cases, with the exception of clown beetles (Histeridae) introduced for control of pests that infest livestock dung, and impacts on pest suppression have been limited, apart from ladybird beetles (Coccinellidae) introduced for the control of scale insect and mealybug pests (Heimpel & Mills, 2017).

While success rates on islands have been suggested to be greater than for mainland locations (Greathead, 1986; Stiling, 1993), due perhaps to reduced biotic resistance from resident natural enemies, the evidence so far has been inconsistent although rather more compelling for New Zealand pasture pests (Goldson et al., 2020). Similarly, although variation in success rates has been observed among continents, it remains unclear why such patterns should occur.

7.4.3 Criteria for Natural Enemy Selection

Introduction

Importation biological control began as a very pragmatic approach to pest management in the 1880s and the majority of projects were based on importations of multiple control agents to increase the likelihood that at least one effective species would establish and provide control of the pest population. Referred to as the lottery model (Denoth et al., 2002), this early empirical approach to importation made little attempt to identify and select control agents with the greatest potential for success. Nonetheless, the success of iconic projects such as the introduction of the vedalia beetle, *Rodolia cardinalis*, from Australia for control of the cottony cushion scale, *Icerya purchasi*, in citrus groves in California in 1888 did capture the attention of population ecologists who sought to explain the scientific basis for success from ecological theory (McEvoy, 2018). Consequently, criteria for selecting the most effective control agents began to be developed and applied to new importation programmes from the start of the 1970s. A reductionist approach that focused on life-history and behavioural traits of natural enemies was used initially as such traits could be incorporated into simple analytical models of parasitoid–host interactions (Sect. 7.3.7) to explore potential impacts of introduced control agents on equilibrium densities of a pest population (Waage, 1990). However, Gutierrez et al. (1994) questioned the reliance of biological control practice on theory, arguing that the latter had contributed little either to increasing the rate of success or to an understanding of the reasons for failures (see also Waage & Mills, 1992; Barlow, 1999; Heimpel & Mills, 2017; Segoli et al., 2023). Similarly, Waage (1990) argued that a more holistic approach to agent selection is needed to better integrate the specific traits of individual control agents into the population ecology of a pest. Subsequently, the safety of the traditional *ad hoc* approach to importation projects was called into question (Howarth, 1991; Simberloff & Stiling, 1996) and the need to limit the introduction of control agents to those that are sufficiently host specific so as not to put non-target species at risk was recognised (Waage & Mills, 1992; Barratt et al., 1997).

During the exploration phase of a biological control programme, a decision will need to be made as to whether the natural enemies are to be collected from the pest species or from other, taxonomically closely related, species. The theory of new associations (Hokkanen & Pimentel, 1984) states that natural enemy–pest interactions will tend to evolve towards a state of reduced natural enemy effectiveness, and that natural enemies not naturally associated with the pest (i.e., species presumed to be less coevolved with the target pest), either because they do not come from the native area of the pest or because they come from a related pest species, may prove more successful in biological control. Hokkanen and Pimentel (1984) analysed 286 successful introductions of biological control agents (insects and pathogens) against insect pests and weeds, using data from 95 programmes, and concluded that new associations were 75% more successful than old associations. However, the validity of

this conclusion was called into question by a more refined analysis of the BIOCAT1992 database (Waage, 1990). The latter showed that the probability of establishment of new-association natural enemies was only half that of old-association natural enemies, and that there was no evidence that the outcome for those that did establish was any more successful. This evidence combined with the added risk of new-association natural enemies, due to a greater host range, has limited more widespread consideration of this approach for the selection of natural enemies in biological control. Nonetheless, there have been some very successful examples of natural enemy introductions using new associations (Heimpel & Mills, 2017). The tarnished plant bug, *Lygus lineolaris*, is a good example of the successful control of a native pest in North America by an exotic new-association parasitoid *Peristenus digoneutis*, with the outcome that nymph densities in alfalfa were reduced by 75% (Day, 2005). Consequently, the potential usefulness of new associations should continue to be considered (Waage & Mills, 1992).

If only a fraction of the natural enemy complex of a pest can be used for importation biological control, it is essential that the most effective and least 'risky' species can be identified and selected from among the candidates available (Waage & Mills, 1992; Mason et al., 2008). In addition, as 74% of the natural enemy species used in importation biological control have either failed to establish in the target region or failed to impact the invasiveness of the target pest (Cock et al., 2016), ecological theory still has the potential to make significant contributions to the selection of control agents for use in future programmes (Wajnberg et al., 2016; McEvoy, 2018; Mills, 2018; Segoli et al., 2023). Below we consider some of the reductionist and holistic traits that should be considered.

Behavioural and Life-history Traits in the Selection of Biological Control Agents

Introduction

We discuss below attributes of natural enemies considered to be among the most desirable for biological control, based on theoretical modelling, practical considerations and past experience. Many of these traits translate to parameters used in analytical parasitoid–host or predator–prey population models (Sect. 7.3.7). We start with reductionist traits associated with the functional response to host density, and then move on to those associated with the numerical response. Finally, we address host or prey specificity, and consideration of climatic matching and ease of rearing. We should not expect to find natural enemies that have all of the desirable attributes and we should anticipate that there will likely be trade-offs among them (Mason et al., 2008).

Traits Associated with the Functional Response

Whether or not hosts can be attacked by a natural enemy often depends on the physical accessibility of the host and the fraction of the host population that is protected from natural enemy attack within a spatial or temporal refuge (Berryman & Hawkins, 2006). Refugia can be thought of in two ways with respect to biological control. On the one hand, a refuge reduces the fraction of the pest population that is attacked, reducing pest suppression. On the other hand, a refuge facilitates stability of the natural enemy–pest interaction over time because it precludes extinction of the entire pest population. While some refuge for the pest may be needed for persistence of the interaction, if the refuge is large, most of the pests can escape from the natural enemy, and consequently the impact of the natural enemy on the pest population will be small, no matter what other attributes it may possess (Hochberg & Holt, 1999; Mills, 2001; Murdoch et al., 2003; Gutierrez et al., 2008).

In this context, Hawkins and Cornell (1994) evaluated the importance of a host refuge from parasitism as a criterion for the selection of more effective parasitoid species for use in biological control programmes. They posed the question of whether a parasitoid that achieves a high rate of parasitism in its native range could be used as a measure of the extent of the host refuge from parasitism and be used to predict the likelihood of success in importation biological control. Using the BIOCAT1992 database, they obtained

a positive correlation between maximum level of parasitism in the pest's region of origin, and the degree of success in importation biological control, indicating that maximum parasitism rate can be used as a reverse measure of the fractional size of the host refuge from parasitism. In addition, they found that a cut-off exists at maximum parasitism rates of approximately 35%; below this level an introduced parasitoid very rarely achieves economic success as the outcome of importation biological control (Hochberg & Holt, 1999).

Another important feature of the functional response is the asymptotic limit to the *per capita* capacity of a natural enemy to attack prey at higher prey densities. This *per capita* limit represents the maximum rate of consumption by predators as determined by the effects of satiation, and the maximum rate of host attack as determined by the egg load (lifetime fecundity divided by mean clutch size) or attack capacity (the maximum number of hosts that a parasitoid can attack in its lifetime) of a parasitoid. These rates are sometimes referred to as the 'killing power' of a natural enemy (Mills, 2005a). Stage-specific daily consumption or parasitism rates can be obtained either from field observations for predators (van den Berg et al., 1997; Latham and Mills, 2010) or from laboratory studies for both predators and parasitoids (Ro & Long, 1998; Hallet et al., 2014) and can be used to inform the relative *per capita* capacities of natural enemy species for pest population suppression.

Other components of the functional response that have been considered of potential importance include a high search rate, the shape of the response and environmental conditions. The search rate defines the speed of approach to the asymptotic *per capita* limit and is often influenced by the strength of the response of a natural enemy to infochemical cues associated with the pest or its feeding activity. Despite the intuitive appeal of a high search rate, Kimberling (2004) found no evidence that search rate was associated with success, based on the historical record of introductions to the USA. However, Gutierrez et al. (1993) demonstrated the importance of parasitoid search rate using a simulation model to analyse the factors influencing the successful biological control of the cassava mealybug *Phenacoccus manihoti* in Africa (Sect. 7.3.8). Of the two introduced parasitoids, *Anagyrus lopezi* finds mealybug colonies five time faster than *A. diversicornis*, and its greater search rate proved to be instrumental in the suppression of mealybug population abundance in the model.

Sigmoid functional responses (Sect. 1.14) are potentially stabilising at low pest densities, which is advantageous, because they result in density-dependent parasitism or predation. Using the BIOCAT1992 database, Fernández-Arhex and Corley (2003) tested for, but were unable to detect, a relationship between the form of the functional response (Type II *versus* Type III) and success in importation biological control. Although prey and predator densities are considered the most important factors that affect *per capita* consumption rates by natural enemies (Arditi & Ginzburg, 2012; Garay et al., 2014), environmental conditions including floral resources (Lee & Heimpel, 2008) and climate (Rall et al., 2012) can also play a role. For example, temperature can influence the consumption rate of mosquito larvae by the notonectid predator *Anisops sardea* (Fig. 7.22), particularly at higher prey densities, and thus could also affect comparisons of prey kill rate among natural enemy species. Rochat and Gutierrez (2001) and Gutierrez et al. (2008) have also emphasised the importance of weather-driven physiologically based functional and

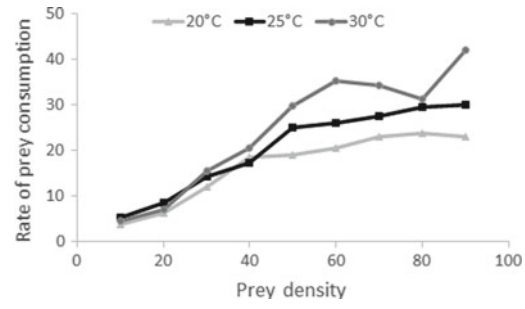

Fig. 7.22 Mean rate of consumption of mosquito larvae (*Anopheles stephensi*) by the predatory hemipteran *Anisops sardea* at increasing temperatures (modified from Mondal et al., 2017, with permission)

numerical responses in models used to explain successes and failures in importation biological control programmes.

Traits Associated with the Numerical Response

The numerical response of a natural enemy represents the change in predator/parasitoid density as a function of increasing prey/host density and includes two components, a reproductive response (a change in the rate of predator/parasitoid reproduction, development or survival), and an aggregative response (a change in the number of predators/parasitoids moving into a prey patch) (Hassell, 2000a, 2000b). As one aspect of the reproductive numerical response, it has frequently been suggested that high fecundity is a necessary attribute for a natural enemy to be able to respond effectively to changes in the abundance of a pest (Beddington et al., 1978; Waage, 1990), but this has seldom been explored in the context of selection of agents for importation biological control. Lane et al. (1999) showed that in their model (which incorporated parasitoid fecundity limitation, a refuge from parasitism, and a density-dependent host population growth function), a high fecundity should provide a greater degree of host suppression and stable control of a host population over a wider range of parameter space. They also found empirical support for a correlation between high fecundity and success in biological control from the BIOCAT1992 database, which revealed a positive correlation for parasitoids introduced against Lepidoptera, although not for parasitoids introduced against Hemiptera (Homoptera). Mills (2001) noted that parasitoids may either be solitary (one offspring develops from each host) or gregarious (multiple offspring develop from a host) and through modelling, similarly identified parasitoid attack capacity as a major factor in pest suppression, irrespective of whether transient or equilibrium dynamics best represent the real dynamics of parasitoid–pest interactions, and that attack capacity was also positively correlated with the probability of success from the BIOCAT1992 database of importations for Lepidoptera. In a similar vein, modelling studies by Heimpel (2000) and Mills (2001, 2006a) have also shown that increased parasitoid brood size can lead to greater suppression of host densities. Even small increases in parasitoid brood size can lead to dramatic reductions in host abundance, and Mills (2001) again found support from the BIOCAT1992 database that gregarious parasitoids were better represented among successes than failures, particularly for Lepidoptera (Mills, 2006a).

As another aspect of the reproductive numerical response, modelling by Godfray and Hassell (1987) in relation to parasitoids, and Kindlmann and Dixon (1999) in relation to predators, has pointed to the role of natural enemy and pest generation times in determining equilibrium levels of host suppression. Godfray and Hassell's (1987) simulations indicate a slight raising of pest equilibrium density when the generation time ratio (GTR, the ratio of the natural enemy's generation time to that of its host or prey) is greater than one, while Kindlmann and Dixon's (1999) simulations reveal that the suppressive effect of a predator is inversely related to the GTR (see Kindlmann & Dixon, 1999, 2001 for a functional explanation). In addition, Mills (2006a) used a simple host–parasitoid model to show that a GTR of 0.5 can reduce the equilibrium density of a pest substantially, and to a much greater extent than parasitoid brood size (Fig. 7.23). Further examination of the BIOCAT1992 database showed that a GTR < 1 was frequently associated with success for homopteran pests, but not lepidopteran pests, while the reverse was the case for parasitoid gregariousness. Thus, the historical record shows that multiple natural enemy generations per host generation is correlated with the success of importation biological control of Hemiptera (Homoptera), whereas gregarious parasitoid development is correlated with success against Lepidoptera (see Mills, 2006a, for possible explanations).

Similarly, Murdoch et al.'s (1987) stage-structured parasitoid-host model (in which either the adults or the juveniles of the pest can be specified as invulnerable to attack from the parasitoid) incorporates a developmental delay in both the host and the parasitoid. The stability of

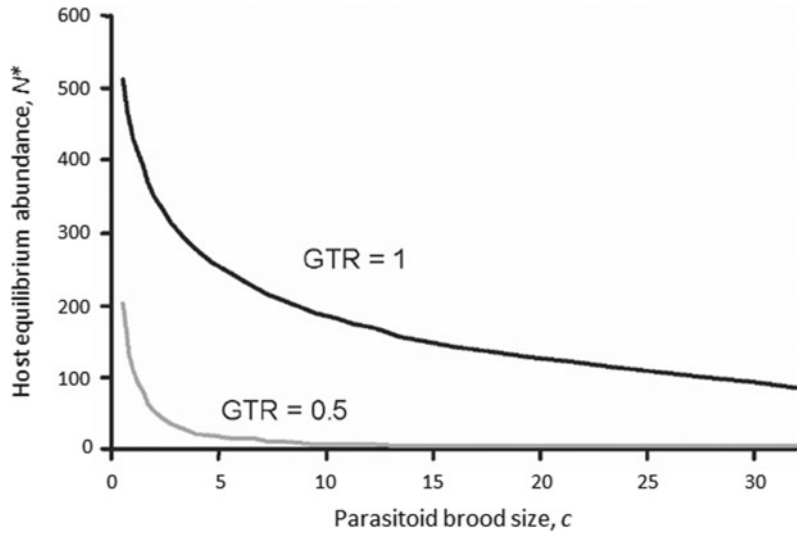

Fig. 7.23 The influence of generation time ratio (GTR) in comparison with parasitoid brood size (c) on the equilibrium density of the host population (N*) assuming a broad window of parasitoid attack (after Mills, 2006a)

this model depends on the length of the parasitoid time lag, relative to the duration of the invulnerable stage. The parasitoid's time lag is destabilising: the longer the developmental period of the parasitoid is relative to that of the host, the more difficult it is to obtain stability. A longer parasitoid development time also leads to exponential increases in the pest equilibrium. Therefore, Murdoch (1990) and Murdoch et al. (2003) considered a short parasitoid development time to be a desirable attribute of a parasitoid species for biological control.

As a final component of the reproductive numerical response, Stouthamer (1993) considered the merits of arrhenotoky (unfertilised eggs develop into males) and thelytoky (unfertilised eggs develop into females) in parasitoids on both genetic and ecological aspects of their success in importation biological control. Some of his conclusions were that: (1) arrhenotokous species, or 'strains', will be able to adapt more rapidly to global change. If environmental conditions in the area of introduction are different from those in the native range, arrhenotokous parasitoids may have the advantage; (2) assuming that a thelytokous strain and an arrhenotokous strain produce the same number of progeny, the thelytokous strain will (all else being equal) have a higher rate of population increase, and suppress pest populations to a lower level of abundance; and (3) arrhenotokous species, or strains, must mate to produce female offspring; therefore, in situations where parasitoid densities are very low, mating success may be compromised (an Allee effect, a positive relationship between individual fitness and population size at low densities; Kramer et al., 2018). Thelytokous parasitoids should therefore be better colonisers.

In support of the first of these conclusions, the declining effectiveness of *Microctonus hyperodae*, a parasitoid of Argentine stem weevil, *Listronotus bonariensis*, in New Zealand pastures, provides evidence that thelytoky may limit the capacity of an introduced parasitoid to coevolve with its host, which appears to have allowed the pest to evolve resistance through enhanced evasive behaviour (Tomasetto et al., 2018). In addition, modelling revealed that when hosts and parasitoids have divergent reproductive strategies that do not generate equal amounts of genetic variation, host resistance to parasitism can readily evolve (Casanovas et al., 2019). Similarly, in support of the third conclusion, a reaction–diffusion model comparing arrhenotokous parasitoids with sexually reproducing diploid ones predicted that haplodiploidy permits successful establishment in parasitoid populations that are 30% smaller: diploid populations suffer more from an Allee effect (Hopper & Roush, 1993).

Mills (2000) outlined a post-introduction protocol for assessing the influence of mating on parasitoid establishment (see also Hopper, 1996). It includes releasing cohorts of increasing size (released as mature pupae) in spatially replicated locations, then dissecting the resulting female parasitoids at host patches, to assess whether they have been inseminated or not (Sect. 4.4).

The aggregative numerical response is influenced by how natural enemy individuals respond to the patchy distribution of hosts in a spatially fragmented environment. The aggregative response provides a linkage between the foraging behaviour of natural enemies and the population dynamics of their hosts (Ives, 1995). It likely depends on dispersal ability, ability to respond to infochemicals associated with their host or plant damage, and a series of decisions that are made as individuals move among patches of hosts. From a theoretical perspective, Mills and Heimpel (2018) discuss how the interference ideal free distribution model (Krivan et al., 2008) provides a simple representation of the optimal distribution of natural enemies among patches based on a balance between the positive effect of host density and the negative effect of interference competition. Although the model assumes that natural enemies have 'ideal' knowledge of the host density and quality of each patch and are 'free' from any costs associated with travel between patches, predicted optimal distributions of enemies among patches seem robust to violations of these assumptions (Griffen, 2009). The optimal behaviour for natural enemies is to aggregate in patches of higher host density and density-dependent aggregation leads to greater host suppression in spatially explicit models (Murdoch et al., 2003; Bianchi et al., 2010). Mills and Heimpel (2018) discuss the similarities between spatial models of aggregation based on the ideal free distribution (Sutherland, 1983) with those of natural enemy load based on the resource concentration hypothesis (Stephens & Myers, 2012). The latter models suggest that the greatest degree of temporal host suppression may be associated with spatial distributions of natural enemies that provide an exact match to those of their hosts (producing a constant natural enemy load among patches) or slight undermatching (producing a slight decline in natural enemy load for patches with higher host densities). Despite the theoretical attention that the aggregative numerical response has received, there is very little empirical information relating to the spatial patterns of attack by natural enemies used in biological control (Mills, 2000). An exception comes from the parasitoids attacking California red scale (*Aonidiella aurantii*), as Murdoch et al. (1996b, 2006) carried out experimental manipulations of both the distribution and the abundance of the scale insect on individual citrus trees. From this study the authors concluded that the spatial heterogeneity in parasitoid attack that characterises this parasitoid-pest system did not account for either local stability or successful reduction in scale abundance. Mills and Heimpel (2018) also suggest how the foraging responses of natural enemies to the spatial distributions of their hosts can be examined experimentally for both past and future importation biological control programmes.

More generally, if a natural enemy has a high ability to disperse (either as an adult or as an immature stage within the host), then it can be expected to spread rapidly from the initial release point. Thus, fewer resources (time, money) may need to be invested in large numbers of point releases over a region to ensure that the natural enemy becomes established over a wide area. Wilson and Hassell (1997) have shown, through modelling, that demographic stochasticity increases the probability of extinction of small local populations and that, because of this, higher dispersal rates are required to ensure persistence of the metapopulation. Another reason for favouring high dispersal capability in importation biological control agents is that it can minimise a time-delay in re-invasion of areas where the enemy has, for reasons of local instability, become extinct; a significant delay can allow the pest population to reach undesirable levels. High rates of parasitoid dispersal have also been shown to be advantageous in the context of biological control where insecticide application also occurs (Keaser et al. 2023). On the other

hand, Heimpel and Asplen (2011) point out that high rates of dispersal can make founder populations of natural enemies susceptible to Allee effects and decrease the probability of establishment. Using modelling, Kean and Barlow (2000) show that a high rate of dispersal can be a significant drain on the rate of increase of a local population. Goodsman and Lewis (2016) also derive an expression to estimate the minimum founding population size required to ensure local establishment in spite of dispersal and a strong Allee effect. In contrast, too low a rate of dispersal could lead to very localised establishment and the potential for inbreeding depression (Heimpel & Asplen, 2011). Consequently, Heimpel and Asplen (2011) argue that a Goldilocks hypothesis of an intermediate level of dispersal is optimal as it maximises the probability of establishment and appropriate spread of an introduced control agent. This could explain why Kimberling (2004) found no correlation between dispersal ability and the success of historical introductions in the USA. Heimpel and Asplen (2011) also suggest approaches for screening candidate agents for dispersal traits and for manipulating dispersal rates at the time of field release. In addition, techniques for studying dispersal by natural enemies are discussed in Sect. 6.2.11.

Host Specificity and Hyperparasitism

One explanation for the poor performance, overall, of predators compared with parasitoids in importation biological control is their tendency to be more polyphagous (Kimberling, 2004). Among introductions of coccinellids, success rates have been higher for monophagous species than for polyphagous ones (Dixon, 2000). It is argued that a pest cannot be maintained at low equilibrium populations by a polyphagous predator or parasitoid, as the natural enemy will concentrate on the more abundant alternative host or prey species. However, as Murdoch et al. (1985) point out, a polyphagous natural enemy can survive in the absence of the pest in the event of the latter's local extinction, and it can therefore be ready to attack the pest when it re-invades. For this reason, polyphagy may not be as undesirable an attribute in importation biological control as it is commonly assumed to be, although polyphagous natural enemies pose greater risks to non-target organisms (Kimberling, 2004; Sect. 7.4.4).

In addition to host specificity, foreign exploration studies have generally focused on identifying and excluding hyperparasitoids from consideration as biological control agents. Theoretical models remain equivocal about the role of hyperparasitism in biological control, with both the disruption or the stabilisation of host–primary parasitoid interactions being possible outcomes (Rosenheim, 1998). In addition, experimental evidence for disruptive effects of hyperparasitism on the success of biological control also remains limited (Rosenheim, 1998; Sullivan & Völkl, 1999; Schooler et al., 2011). Nonetheless, the disruptive nature of hyperparasitism remains the prevailing view and, more recently, consideration has been given to exploitation of chemical ecology for the management of hyperparasitoids (Cusumano et al., 2020) and to hyperparasitoids as potential targets for biological control (Tougeron & Tena, 2019).

Climatic Matching

The optimum range of temperatures or humidities for development, reproduction and survival of a candidate biological control agent may be different from that of the pest, and the natural enemy may either fail to establish or prove ineffective owing to the direct or indirect effects of climate in the region of introduction. The conventional wisdom is that a parasitoid species should be collected from a location in the region of origin where climatic conditions provide an optimal match to those that prevail in the region of introduction (DeBach & Rosen, 1991; Sect. 2.9.3). This view is supported by the database analysis of Stiling (1993) which showed that the climatological origin of parasitoids has a large influence on establishment rate. However, the climatic adaptation criterion should not be rigidly applied: *Anagyrus lopezi*, which successfully controlled cassava mealybug in West Africa, originated from Paraguay, where the climate is very different (Gutierrez et al., 1994;

Neuenschwander, 2001). In addition, more recently this same parasitoid has proved to be equally successful in controlling cassava mealybug across the heterogeneous cassava cropping environments of Southeast Asia (Wyckhuys et al., 2018).

Despite this anomaly, it is generally accepted that climate matching is an important consideration in maximising the potential for success of importation biological control (Hoelmer & Kirk, 2005; Robertson et al., 2008; Mills & Kean, 2010). Having determined the distribution of the target invasive pest in its native region and/or the thermal requirements of a candidate agent, climatic niche models such as CLIMEX (Kriticos et al., 2015, 2021) and MaxEnt (Phillips et al., 2006) offer a practical method for evaluating climatic effects at several of the steps in an importation programme. Based on the known distribution of the target invasive pest the climate-matching tools of CLIMEX can be used to identify locations in the region of origin for foreign exploration that are climatically most similar to those in the invaded region. More sophisticated climatic niche models based on the thermal requirements and tolerances of candidate agents can then be used not only for predicting the prospects for establishment in a target region, but also for identifying when and where to release approved biological control agents to coincide with the seasonality of the target pest and potential risks to non-target species. Tanga et al. (2021) provide an example of using climatic niche models, based on MaxEnt, to identify climatically suitable regions for foreign exploration for parasitoids of the mango mealy bug, *Rastrococcus iceryoides*, in India and suitable areas for parasitoid releases in invaded areas of Africa and Asia. Similarly, using CLIMEX, Avila and Charles (2018) provide an example of how to predict the geographic range of the exotic parasitoid *Trissolcus japonicus* in New Zealand, and its potential risk to non-target species.

Mills (2000) recommended investigating the role and importance of climatic matching experimentally, post importation, by either releasing fixed numbers of parasitoids from a single climatically characterised founder population along a climatic gradient in the target region, or using unique genetic markers (Sect. 3.2.2) to identify different geographic strains of a single parasitoid species and to release them in combination at a series of climatically different locations in the target environment. The latter method can allow the success of local establishment to be related to the degree of climatic match between original and target localities for each strain. Fischbein et al. (2019) also used MaxEnt to demonstrate the benefits of climate matching for predicting both success and failure of establishment of parasitoids introduced to South America, Africa and Oceania for biological control of the forest pest *Sirex noctilio*. Climate alone provided accurate predictions for two of its parasitoids, *Ibalia leucospoides* and *Megarhyssa nortoni*, but other factors may also limit the establishment of *Rhyssa persuasoria* in Brazil and Patagonia (Fig. 7.24).

Ease of Handling and Culturing

While not a trait for consideration in the selection of control agents, Greathead (1986) concluded, from an analysis of the BIOCAT database, that the most important factors in the selection of natural enemy species for use in importation biological control programmes have, perhaps, been ease of handling and availability of a technique for culturing the insects. The case of biological control of the mango mealybug, *Rastrococcus invadens*, is an illustration of how ease of rearing can influence selection. Two encyrtid parasitoids, *Gyranusoidea tebygi* and *Anagyrus* sp., were being considered for introduction into West Africa. Despite the latter species being the dominant parasitoid in rearings from field-collected mealybugs in India, the former species was selected as the first candidate for introduction, owing to the ease with which it could be cultured (see Waage & Mills, 1992 for a discussion). A reason given by Waage (1990) for the more extensive use of Ichneumonidae compared with Tachinidae in programmes aimed at

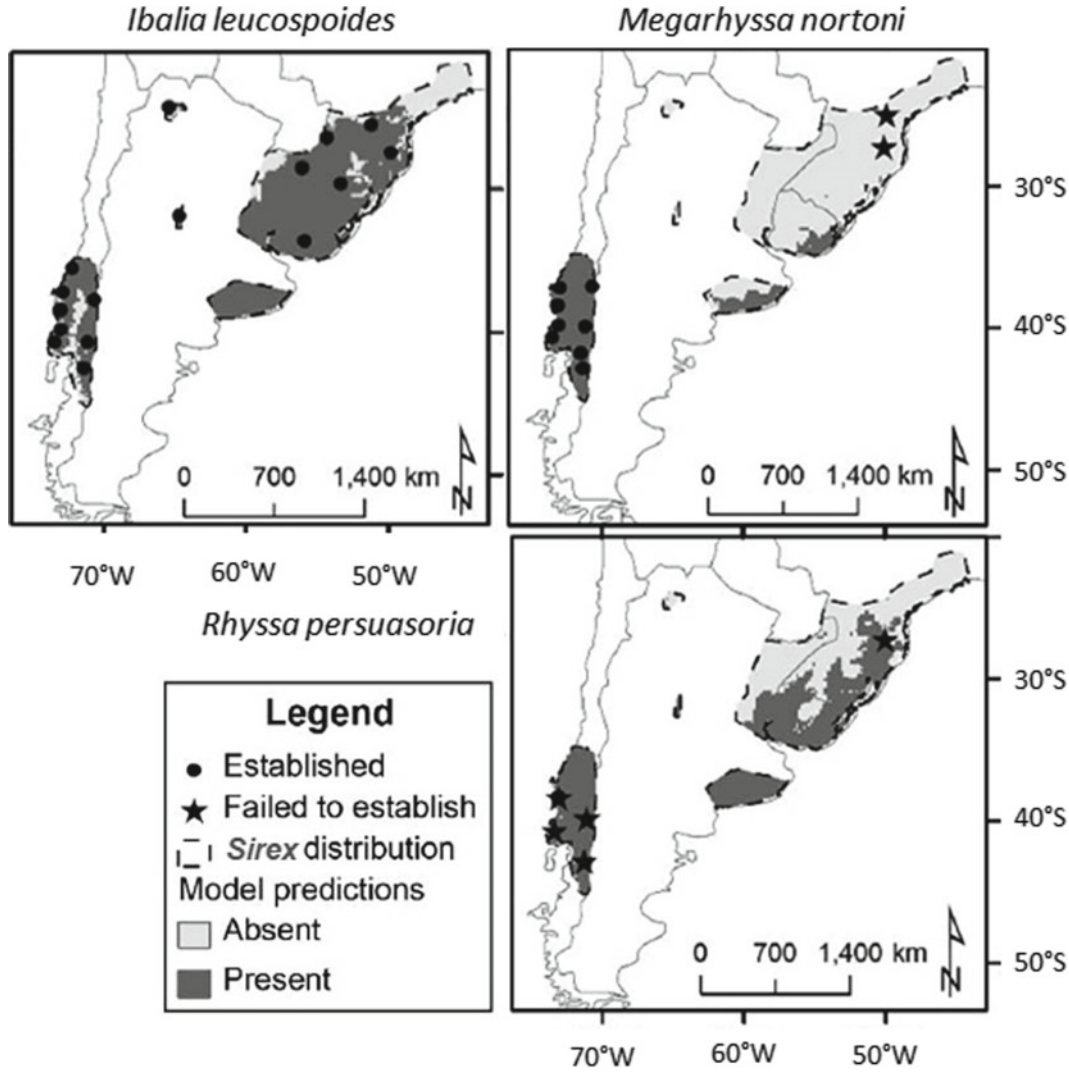

Fig. 7.24 Predicted geographic range in South America of the three parasitoid species introduced to the southern hemisphere for importation biological control of the wood wasp *Sirex noctilio* showing the distribution of the wood wasp (dashed line), model predictions for the presence (dark grey) and absence (light grey) of the parasitoids, and locations where they either established (black circles) or failed to establish (black stars) (modified from Fischbein et al., 2019, with permission)

controlling exotic Lepidoptera is the greater difficulty encountered in culturing the latter parasitoids. It is also noteworthy that the ranking of culturable agents for introduction has usually followed the sequence in which they were established in culture (Waage, 1990). In this context, it is important not to eliminate potentially effective agents from consideration just because they are difficult to handle and culture in captivity.

Population and Community Level Considerations in the Selection of Biological Control Agents

Introduction

In contrast to the focus on life-history traits of natural enemies, the holistic approach to the selection of agents addresses the dynamic nature of natural enemy–host interactions (Waage, 1990) and how demographic and genetic

processes can influence the establishment and impact of introduced natural enemies (Mills, 2018). Examples of this approach are presented below.

Collecting Parasitoids from Non-outbreak Areas in the Native Range of the Pest

Selection of agents can begin during the exploration phase of a programme. If an invasive species is known to have outbreaks in its region of origin, then intuitively we would expect that the natural enemy species present during host outbreaks would not necessarily be those best suited to preventing outbreaks and maintaining the pest at low densities in an invaded region (Pschorn-Walcher, 1977; Fuester et al., 1983; Waage, 1990; Waage & Mills, 1992). In contrast, focusing exploration on low-density populations of the invasive pest in its region of origin could provide a more effective approach for selecting the 'best' natural enemy species. Waage (1990) and Waage and Mills (1992) also recommend the use of sentinel host cohorts exposed to natural enemies in the field as a more practical alternative to the challenge of conducting exploration surveys in natural, low-density host populations (see Sects. 6.2.8 and 7.2.3 for methodology).

Selection of Agents in the Context of Density-Dependent Mortality and Vulnerabilities in the Pest Life-cycle

Density-dependent mortality acting later in a pest's life-cycle can influence the contribution of mortality from natural enemies acting earlier on (May & Hassell, 1988). Indeed, if the density dependence is over-compensating, too high a level of parasitism acting early in the life-cycle can lead to increased host population densities later in the life-cycle (van Hamburg & Hassell, 1984; Suh et al., 2000).

In a discussion of augmentative releases of *Trichogramma* against stem-boring Lepidoptera, van Hamburg and Hassell (1984) concluded that the success of a programme will be largely influenced by the level of egg parasitism, the level of the subsequent larval losses, and the degree to which the latter are density dependent. Similar considerations have also been suggested for programmes in which deliberately introduced exotic natural enemies are used for the biological control of invasive pests (Goldson et al., 1994; Abram et al., 2020).

It is also often assumed that parasitism or predation at any stage in the life-cycle of a pest can contribute equally effectively to successful biological control. As indicated above, however, the timing of major density-dependent mortalities in the life-cycle of a pest can compensate for the contribution of parasitism to pest suppression. More generally, it is possible that other specific aspects of the demographic vital rates of a pest could also influence the impact of natural enemies acting at different stages in the life-cycle. One way in which the life-cycles of pests can be screened for vulnerabilities that could maximise the effect of added mortality from introduced natural enemies is through prospective or predictive modelling using stage-structured matrix models (Shea & Kelly, 1998; Mills, 2005b, 2008; Abram et al., 2020). The elements of a matrix model consist of two probabilities for each of the stages in the life-cycle of a pest: one representing the probability of survival and successful transition from one life stage to the next, and the other representing the probability of survival and stasis or remaining within the same life stage, plus the daily *per capita* offspring production during the reproductive phase of the adult stage (Caswell, 2001). The probabilities of transition and stasis are estimated from component vital rates for development and survival at each life stage and the daily offspring production from the realised fecundity, duration of the reproductive phase, and female sex ratio. Elasticity analysis of the resultant model (which estimates the effect of a proportional change in a vital rate on population growth rate) can be used to identify vulnerabilities in the life-cycle. The larger the elasticity, the greater the relative importance of the component vital rate as a contribution to population growth rate. Thus, the life stage or stages with the greatest elasticity for daily survival rate represent vulnerabilities in the life-cycle of the pest where the addition of mortality from an introduced natural enemy would have maximum impact in suppressing pest population growth.

As one of the criteria for the selection of parasitoid species for introduction from Kazakhstan to California, USA, Mills (2005b) used a simple stage-structured matrix model to assess the relative importance of adding parasitism to each of the different stages in the life-cycle of the codling moth, *Cydia pomonella*, as a pest of pome fruit and walnuts. The elasticity analysis of the model identified the cocoon stage as the most vulnerable stage in the life-cycle of this pest and *Mastrus ridens*, a specialist cocoon parasitoid, became the main focus of the biological control programme for codling moth in the western region of the USA where it has become established with parasitism rates of overwintering cocoons reaching 70% in some unsprayed orchards. Other examples of prospective analyses of life-cycle vulnerability for invasive insect pests include light brown apple moth, *Epiphyas postvittana*, and generic stink bugs (Mills, 2008; Abram et al., 2020).

Complementarity and Antagonism in the Reconstruction of Natural Enemy Communities for Invasive Pests

The outcome of interactions among species in a natural enemy community have the potential to be either null, additive, antagonistic or synergistic in their effect on the strength of pest suppression (Letourneau et al., 2009; Hajek and van Nouhuys (2016). In addition, numerous studies have shown that additive or synergistic interactions can be very beneficial in the context of conservation biological control and that complementarity can arise through a number of different mechanisms (Snyder, 2019). In contrast, in the context of importation biological control, there has been far less attention paid to complementarity in the reconstruction of natural enemy communities of invasive pests and rather more to the avoidance of antagonism (Batchelor et al., 2006; Mills, 2006b; Heimpel & Mills, 2017).

There has been an ongoing debate about the benefits of single *versus* multiple introductions and the consequences of interspecific competition among natural enemies in importation biological control (Mills, 2006b). Such competition can extend beyond insect parasitoids to include microbial pathogens with outcomes that can vary from facilitation to competitive exclusion. For example, Hajek and van Nouhuys (2016) found that among the natural enemies introduced for control of gypsy moth in the USA, facilitation can occur between the baculovirus LdMNPV and the larval parasitoid *Cotesia melanoscela*, whereas the fungal pathogen *Entomophaga maimaiga* outcompetes each of the four main larval parasitoids. From a (simple) theoretical perspective, insect parasitoids that interact through exploitative competition cannot coexist and a superior species that is able to drive resource densities to the lowest level will successfully exclude or displace others (Murdoch et al., 2003). As pointed out by Kidd and Amarasekare (2012), however, equilibrium dynamics may never be achieved under field conditions and under shorter-term transient dynamics a superior competitor may not always result in the greatest level of pest suppression. In addition, coexistence can be mediated by several factors that include enemy density dependence and either spatial or temporal niche partitioning among enemies. For example, the breaking of a host refuge from parasitism through the introduction of a second parasitoid species that has low niche overlap with the first is a compelling reason to consider multiple introductions, as theoretical models predict that it can lead to substantial reductions in pest densities (Pedersen & Mills, 2004). The historical record of biological control includes several examples of more effective control of arthropod pests from multiple natural enemies (Stiling & Cornelissen, 2005). In addition, although competitive displacement by a superior competitor has been documented for several importation biological control programmes, it has always led to greater pest suppression (Mills, 2006b). Consequently, the outcome of multiple introductions in biological control is generally considered to be either inconsequential or beneficial, particularly if there is evidence for competitive displacement

(Murdoch et al., 1996a) or niche partitioning among enemy species (Rochat & Gutierrez, 2001; Pekas et al., 2016; Duan et al., 2021).

The main concern with regard to multiple introductions is whether antagonistic interactions between natural enemy species, such as intraguild predation and facultative parasitism, could decrease the efficiency of importation biological control. As discussed earlier in the context of hyperparasitism, however, neither theoretical models nor experimental evidence provide a consistent view of the potential for a disruptive effect of these interactions on the success of biological control (Janssen et al., 2006; Rosenheim & Harmon, 2006; Evans, 2016). A range of different factors can influence the impact of both intraguild predation and facultative parasitism on the outcome of biological control. For example, host preference in a facultative parasitoid for a primary parasitoid over its insect host could tip the balance from a positive or neutral effect to a negative effect on the outcome (Moore & Kfir, 1995). In contrast, Finke and Denno (2002) showed how the structural characteristics of an herbivore's habitat can mediate the effects, upon planthoppers, of intraguild predation by wolf spiders upon mirid bugs. In contrast to structurally simple laboratory 'habitats', more complex habitats increased the combined effectiveness of the predators in suppressing planthopper populations. Finke and Denno's (2002) findings suggest that for importation biological control the dynamic significance of intraguild predation will vary according to both the type of agroecosystem involved and/or the type of habitat management practised.

A combination of facultative hyperparasitism and intraguild predation led Batchelor et al. (2006) to recommend against introduction of the parasitoid *Cephalonomia hyalinipennis* for biological control of coffee berry borer, *Hypothenemus hampei*, indiginous to Mexico, to other regions. Subsequent simulation modelling has also confirmed the likely detrimental effects that this candidate agent would have on the outcome of biological control (Rodríguez et al., 2017; Cure et al., 2020). The general perception remains that restraint should be exercised in using either facultative hyperparasitoids or intraguild predators in importation biological control, and laboratory studies can be conducted to screen for antagonistic interactions before control agents are selected for introduction (Batchelor et al., 2005, 2006; Wang et al., 2019).

Selection of Agents in Relation to Host Plant Quality

To protect themselves from insect damage, plants use both direct defence (nutritional quality, deterrence and toxicity) and indirect defence (herbivore-induced plant volatiles to attract natural enemies), and thus plant quality can play an important role in enemy–host interactions (Verkerk et al., 1998; Hunter, 2003; Peterson et al., 2016). Plant quality effects upon host suppression by natural enemies may in some cases be positive (additive, synergistic) or neutral, but in others may be antagonistic. While many plant defence traits have been lost through crop domestication (Chen et al., 2015), whether the bottom-up effects of host-plant resistance are compatible with efficient top-down biological control cannot be assumed and needs to be taken into consideration in the selection of candidate control agents for importation biological control.

Consequently, there is a strong case for employing multitrophic models in biological control (Gutierrez et al., 1994; Mills & Gutierrez, 1999), given the potential for significant bottom-up effects. For example, using linear multiple regression and marginal analysis of the data from a simulation model for the successful control of the spotted alfalfa aphid *Therioaphis maculata* in California, USA, Gutierrez and Ponti (2013) were able to estimate the relative contributions of the different factors included in the model to suppression of aphid densities. The greatest contributions came from the development of new alfalfa varieties that had greater host-plant resistance to the aphid and the action of native coccinellids. In this example, the host-plant resistance also proved to be compatible with parasitism by the three introduced parasitoids (dominated by *Trioxys complanatus*) that combined to achieve successful control of the invasive aphid.

More generally, the compatibility of host-plant resistance and importation biological control will depend on the strength of the negative effects of direct defences on the pests themselves and their suitability as hosts or prey for natural enemies, and the extent to which changes in indirect defences disrupt the signalling pathways used by natural enemies. Plant quality seems likely to affect potential control agents in different ways and thus greater emphasis on testing for the compatibility of modern crop varieties with available candidate agents should be included among the criteria considered in the selection of natural enemy species for use in importation biological control.

Selecting for Seasonal Synchrony with the Target Pest

Populations of hosts and parasitoids with discrete generations frequently show imperfect phenological synchrony, with the result that some host individuals experience a temporal refuge from parasitism. Compared with perfect synchrony, imperfect synchrony will result in reduced host suppression, although models developed by Münster-Swendsen and Nachman (1978) and Godfray et al. (1994) show that it can stabilise the parasitoid–host population interaction. For example, there is considerable variation in the synchrony of the specialist parasitoid *Cotesia melitaearum* with its host butterfly *Melitaea cinxia* that is driven by cool early spring temperatures (van Nouhuys & Lei, 2004). By basking in the sun, the dark-coloured host larvae can complete their development and escape from parasitism before adult parasitoids emerge from overwintering cocoons. The asynchrony reduces both parasitoid population size and the rate of colonisation of host patches which is likely to be important for the metapopulation dynamics of the host butterfly.

Temporal synchrony can also be lost in novel environments, as demonstrated by the case of synchronisation of the parasitoid *Encarsia citrina* with the susceptible stage of its host the elongate hemlock scale, *Fiorinia externa*. In its native Japan, the scale has two generations a year and parasitism can reach 90% (McClure, 1986). In the United States, however, the number of scale generations varies from one in the north to two in the south, and the introduced parasitoid is poorly synchronised throughout the invaded range (Abell & Van Driesche, 2012). This failure of the programme, due to poor seasonal synchrony (Fig. 7.25), could be due to differences in host-plant quality between tree species in the region of origin and invaded region, again emphasising the value of a more holistic view to the selection of candidate control agents.

Finally, it has frequently been suggested that climate change and extreme climatic events could also lead to imperfect synchrony in enemy–host interactions (Stireman et al., 2005; Hance et al., 2007; Thomson et al., 2010), and Wetherington et al. (2017) demonstrated that even relatively small changes in the severity of extreme climate events can affect emergence times and reduce both parasitism and survival by an egg parasitoid *Oobius agrili* of the emerald ash borer, *Agrilus planipennis*.

Intrinsic Rate of Natural Increase (r_m) and Pest Kill Rate (k_m)

Janssen and Sabelis(1992) were among the first to explore the use of the intrinsic rate of population increase (r_m) as a selection criterion for biological control agents, as it integrates a suite of individual natural enemy traits into a single population-level metric for population growth. In reviewing the biological control programme against cassava mealybug, Neuenschwander (2001), however, concluded that r_m was, in retrospect, a poor predictor of agent effectiveness in that particular case. Modelling by Hochberg and Holt (1999) has shown that r_m (which they estimated from a partial derivative of their host refuge model) is enhanced by a greater searching efficiency, a greater attack capacity (maximum number of hosts attacked over the parasitoid's lifetime) and a greater mean number of parasitoids emerging from a parasitised host. It was also shown that in highly productive environments (high host carrying capacity), it is parasitoid attack capacity alone that determines the conversion of hosts to parasitoids and therefore the transient impact of parasitism on the host

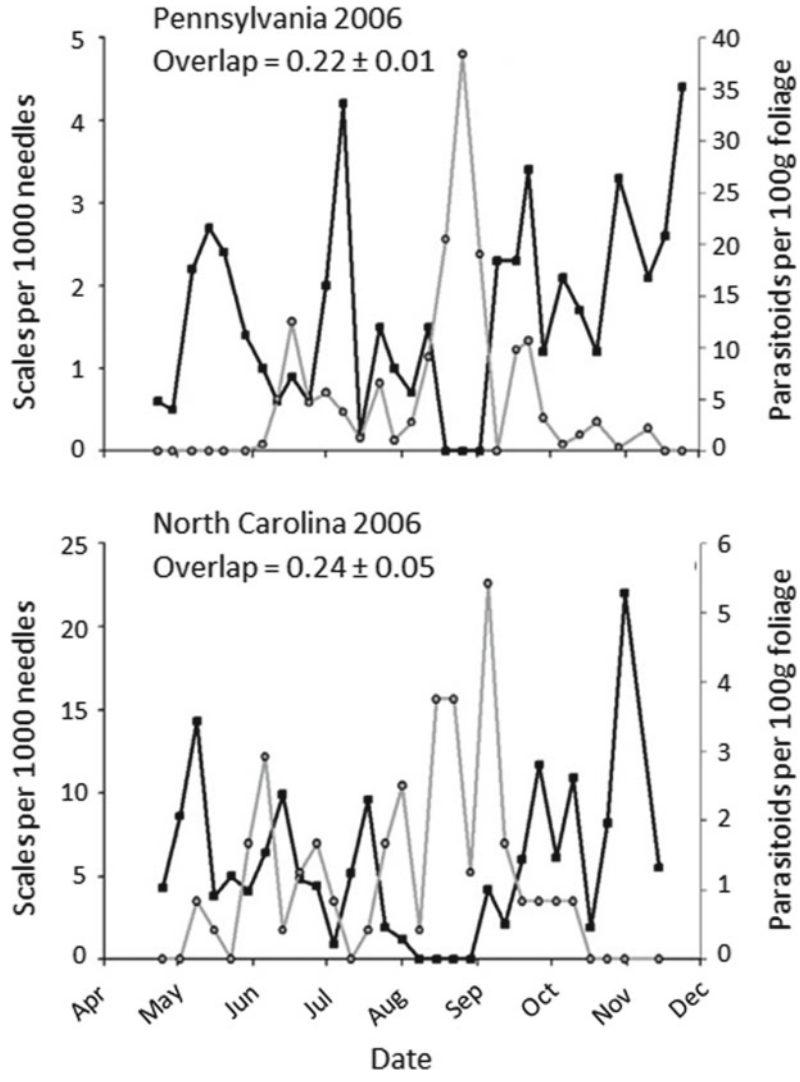

Fig. 7.25 Seasonal abundance of adults of the parasitoid *Encarsia citrina* and of the susceptible stage (second instar) of its host *Fiorinia externa* in Pennsylvania and North Carolina, USA in 2006 showing the limited degree of overlap in seasonal synchrony (modified from Abell & Van Driesche, 2012, with permission)

population. If the brood sex ratio is biased towards females (as is often the case for gregarious parasitoids, Sects. 1.11 and 5.4), then a gregarious species will have a higher population growth rate than a solitary species with the same fecundity (Mills, 2001).

Although r_m can be used as a comparative measure of the potential impact of many solitary parasitoids, as pointed out by van Lenteren et al. (2019), it is less applicable to predators or to parasitoids that are either gregarious or cause additional host mortality through destructive host feeding or stinging. For these latter categories of biological control agents, r_m provides only a measure of the capacity for population growth and not their capacity to kill pests. Alternatively, the pest kill rate (k_m) can be estimated by replacing age-specific fecundity with an age-specific kill rate to provide a better estimate of potential impact for a biological control programme (van Lenteren et al., 2021). A comparison of pest kill rates for six predator and seven parasitoid species as candidate control agents for the South American tomato moth, *Tuta absoluta*, identified the predator *Nesidiocoris tenuis* and the parasitoid *Trichogrammatoidea bactrae* as potentially the most effective species for importation biological control.

One important advantage of pest kill rate is that the estimated impact of parasitism can be extended to include host mortality from destructive host feeding and stinging as well as host mortality from parasitism. In addition, it effectively integrates daily host or prey kill rates with the amount of time that the natural enemy spends in each life stage. Using models and the BIOCAT1992 database, Jervis et al. (1996) questioned whether destructive host feeding is a desirable attribute for a biological control agent. Although modelling suggested no benefit with regard to establishment rate or suppression of host abundance, the historical record revealed that destructive host feeding in parasitoids does lead to a slight improvement in establishment rate and a greater success rate than for non-host feeding parasitoids (Jervis et al., 1996). Thus, as an additional source of mortality, destructive host feeding or stinging does appear to be an important attribute to consider for the selection of biological control agents. While the estimation of pest kill rate captures all of the direct effects of natural enemy impacts on a pest population, it does not include the indirect non-consumptive effects that result from prey responses to the threat of natural enemy presence (Sect. 7.2.5), which can in some cases be as strong as the direct consumptive effects (Buchanan et al., 2017). The strength of indirect effects can differ between natural enemy guilds, and also between predator species, but quantifying these effects on an age-specific, or even stage-specific, basis poses a considerable challenge and has yet to be taken into consideration in the selection of agents for use in importation biological control.

7.4.4 Non-target Effects

Introduction

There has been increasing concern over the risks posed by biological control, especially importation biological control, to natural biodiversity, and since the 1990s numerous studies and reviews of the risks of natural enemy introductions have been conducted (Heimpel & Cock, 2018). Protocols for risk assessment were developed for weed biological control in the 1970s (Wapshere, 1974; see next section below) and have proved to be remarkably successful (Hinz et al., 2014; Paynter et al., 2018). However, no such protocols for arthropod biological control were considered until the 1990s (Van Driesche & Hoddle, 1997), and further developments have continued since that time (Van Driesche & Reardon, 2004; van Lenteren et al., 2006; Heimpel and Mills, 2017; Paynter & Teulon, 2019). The risk from introduced natural enemies can be either direct, due to consumption of non-target species (Lynch et al., 2002), or indirect and mediated by complex interactions within the target community (Messing et al., 2006). Direct risks from introduced natural enemies can readily be assessed through laboratory host-specificity tests of the ability of a candidate control agent to use a non-target host or prey species (including resident natural enemies as well as herbivores). In contrast, indirect risks to food webs and ecosystems from introduced natural enemies are much more difficult to assess even though they are known to be significant in some instances (Heimpel & Cock, 2018).

The evidence for harmful ecological impacts from natural enemy introductions is variable in quality, ranging from anecdotal to relatively quantitative (Lynch et al., 2002). Although there have been some notable examples of negative effects (Van Driesche & Hoddle, 2017) many of them stem from the early period of importation biological control from 1880 to 1960, with direct non-target effects appearing to have stopped after the 1960s (Heimpel & Cock, 2018). There has also been little evidence for host range expansion by natural enemies following introduction (Wright & Bennett, 2018). While a focus on reducing the risks of importation biological control since the 1970s has resulted in a decline in the number of natural enemy introductions worldwide (Cock et al., 2016), it has proved to be beneficial in improving the practice of importation biological control, with particular emphasis on the need for careful monitoring and safety. As regulators in most countries now require risk assessment prior to approval of natural enemy introductions, here we will focus on the

approaches and methods developed for assessing host specificity of arthropod biological control agents and for prediction of ecological impacts.

Host-Specificity Testing to Minimise Direct Non-target Effects

The first step is to choose which non-target species to test. The centrifugal phylogenetic testing method developed by Wapshere (1974) for natural enemies of weeds exposes a candidate biological control agent to a sequence of test plants from those that are most closely related to the target weed to those belonging to successively most distant taxa. This testing method has also been adopted for natural enemies of arthropod pests (Kuhlmann et al., 2006). Important differences, however, are that the range of non-target species available for host-specificity testing of entomophagous species can be extensive and their phylogenetic separation may not be as well understood. In addition, the phylogenetic signal can be weaker for entomophagous insects than for insect herbivores, while host habitat (e.g., leafminers) and other forms of ecological specificity can influence the host range of some species (Messing, 2001). The number of non-target species selected for host-specificity testing of entomophagous insects is fewer than for insect herbivores, can be reduced through initial field surveys to assess host ranges in the region of origin (Kuhlmann & Mason, 2003), but can still often average more than ten (Kuhlmann et al., 2006). The approach is then to expose each non-target species (on its host plant) in turn to a candidate control agent to assess (1) the proportion of hosts or prey attacked, (2) the proportion of hosts parasitised in the case of a parasitoid, and (3) the suitability of the host or prey for supporting successful development and reproduction (van Lenteren et al., 2006). In addition, Paynter and Teulon (2019) suggest that the relative performance of candidate control agents on non-target and target hosts in laboratory host-specificity tests should be considered for potential prediction of the risk of non-target effects in the field.

A number of difficulties can arise in the interpretation of host-specificity tests as no-choice laboratory tests, which are used to assess the physiological host range (the set of non-target species that support development), often overestimate the potential risks of candidate control agents (van Lenteren et al., 2006). Consequently, large arena choice tests and olfactometer studies are also recommended to assess better the ecological host range (the set of non-target species used in the field) of a candidate control agent (Wyckhuys & Heimpel, 2007; Murray et al., 2010). When using parasitoids in choice tests, however, kairomones from the target pest can often result in the non-target species being attacked even though it would not be used as a host under field conditions (van Lenteren et al., 2006).

Other methodological considerations for host-specificity tests include the physiological state of the candidate control agent and what statistical tests to use for analysing host-specificity data. With regard to physiological state, individuals that are hungry and in the case of parasitoids, those that are time limited with high egg loads, are more likely to accept low-quality hosts or prey (Withers & Browne, 2004). Although some studies have not found a significant influence of physiological state on host acceptance in host-specificity tests (e.g., Jenner et al., 2014), it is still recommended that physiological state be taken into consideration. Appropriate choices of statistical analyses for host-specificity tests are also essential for correct interpretation of the data and this has been reviewed by Withers et al. (2013).

Assessing Indirect Non-target Effects

Indirect effects of biological control agents on food webs (Sect. 6.3.12) and ecosystems have rarely been assessed due to the difficulty of quantifying complex interactions. Messing et al. (2006) provide a framework of possible indirect interactions and discuss the observational and experimental approaches that might help to define the role of a candidate biological control agent within a local ecological community. Memmott (2000) points out that quantitative food webs could be a useful tool for a retrospective analysis of indirect non-target effects in

biological control and discusses approaches and methods. Although such food webs are purely observational studies, they can be used to quantify the extent of interaction between a biological control agent and the members of a native community and to generate hypotheses about potential impacts of the agent on the population dynamics of non-target members of the community. It has also been suggested that quantitative food webs could be used as a prospective tool prior to introduction of a biological control agent (López-Núñez et al., 2017). Such an approach is currently difficult to implement but is likely to become more practical in the future as next-generation sequencing technologies (Sect. 3.2.2) facilitate the study of both diet breadth and food web interactions under field conditions (Gonzalez-Chang et al., 2016). A less expensive option is to use a qualitative food web to identify direct interactions with other species in the food web and to predict which non-target species may be at indirect risk (Todd et al., 2021). The latter approach has been used to explore potential indirect non-target effects for two parasitoids in New Zealand; *Cotesia urabae* released for control of the eucalyptus defoliator *Uruba lugens*, and *Trissolcus japonicus* approved, but not yet released, for control of the brown marmorated stink bug, *Halyomorpha halys* (Todd et al., 2021).

7.4.5 Natural Enemy Release and Evaluation

Once approved for field release, a selected biological control agent must be reared, transported to the field and openly released. From studies on invasion biology, it is generally agreed that the probability of establishment increases with propagule pressure, defined as the size and number of introductions made (Simberloff, 2009). Exactly how many individuals to release at each site and how many sites are needed, however, remain open questions. In an analysis of the historical record of biological control, Hopper and Roush (1993) found that establishment from single releases has been more successful when more than 100 individual parasitoids belonging to species of Ichneumonoidea were released or more than 1,000 individuals belonging to the Chalcidoidea or the Tachinidae. Models developed to address the trade-off between conducting more small releases *versus* fewer large releases have been inconclusive, leading to a recommendation that a range of release rates be used for initial releases and that information gained from these be used to optimise later releases (Shea et al., 2002). In addition, Fauvergue et al. (2012) discuss the demographic and genetic processes that influence small founder populations and opportunities for improving the practice of importation biological control.

Once a biological control agent has been released and established, the success of the importation programme should be assessed, but unfortunately this is often neglected due either to lack of financial resources or personnel (Heimpel & Mills, 2017; Segoli et al., 2023. Monitoring the abundance and spread of introduced biological control agents can be carried out using a variety of techniques that are detailed in Sect. 7.2. One recent study compared three different sampling methods (sentinel logs, debarked logs and pan traps) to monitor the establishment and spread of two larval parasitoid species released for control of the emerald ash borer, *Agrilus planipennis* (Rutledge et al., 2021). All three methods detected both species; the use of sentinel logs was the most efficient method for detecting *Tetrastichus planipennisi*, whereas debarking the lower 2 m of ash trees was a more efficient method for detecting *Spathius galinae,* and setting out yellow pan traps was the least efficient method for both species.

In addition to monitoring establishment and spread, the goal of importation biological control is to assess the ecological success of an introduced biological control agent in suppressing pest densities and the economic value of the programme. A change in pest density can be documented through 'before and after sampling', but confirmation of the contribution of the biological control agent to pest suppression requires the use of life-table analysis (Bellows & Van

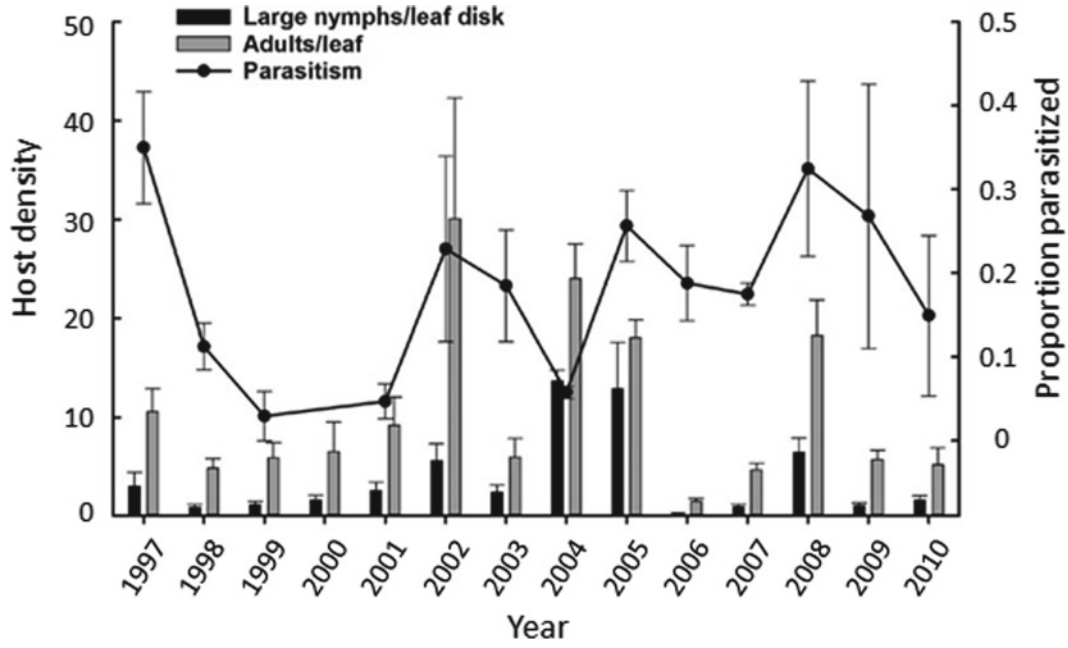

Fig. 7.26 These field data show the absence of a relationship between seasonal mean densities of *Bemisia tabaci* nymphs (black bars) and adults (grey bars) and the marginal rates of parasitism (black line) by the introduced parasitoids *Encarsia sophia* and *Eretmocerus* sp. in Arizona cotton fields. Error bars are ± 1 SE (modified from Naranjo, 2018)

Driesche, 1999; Duan et al., 2014; Sect. 7.3.4), exclusion techniques (Luck et al., 1999; Sect. 7.2.2) or the use of population models (Gutierrez et al., 1994, 2008; Murdoch et al., 2006; Sect. 7.3.7). For example, in a retrospective assessment of the importation biological control programme for *Bemisia tabaci*, Naranjo (2018) used a combination of matrix models, life tables and life-table response experiments to quantify the cause of the decline in abundance and status of this pest in Arizona cotton. This approach revealed that the use of selective insecticides promoted greater populations of native generalist predators in cotton with an associated increase in the mortality of the immature stages of *B. tabaci*. In contrast, there was no improvement in biological control from the establishment of two introduced parasitoids, *Encarsia sophia* and *Eretmocerus* sp. from Ethiopia, with no relationship between host density and marginal parasitism rate (Fig. 7.26). The two parasitoids contributed an average level of 20% parasitism, which is below the threshold found necessary for success by Hawkins and Cornell (1994). The economic value of ecological successes in importation biological control has only rarely been estimated, but Naranjo et al. (2015) summarise the information available and provide a guide to methods and analytical approaches for economic valuation of biological control outcomes for arthropod pests.

7.5 Conclusion

In this chapter we have discussed the established methods as well as recent advances made in quantifying predation and parasitism, evaluating the role played by natural enemies in the dynamics of host populations, and selecting the most appropriate control agents for use in importation biological control. Some of the most significant advances have focused on natural enemy impact assessment such as: (1) introduction of next-generation sequencing as a methodology for detection of predation and

parasitism; (2) increased recognition of non-consumptive as well as consumptive effects of predators and parasitoids; (3) landscape scale assessment of the effectiveness of biological control; (4) development of semi-discrete hybrid models to improve our understanding of the dynamics of host–parasitoid interactions; (5) application of more sophisticated statistical models to analyse time-series data collected from the field; and (6) use of improved methods to predict the success and safety of introduced biological control agents. This focus on natural enemy impact assessment stems from a desire to develop a more robust ecological framework for pest management and to increase the level of confidence among managers in the reliability of biological control. Although important advances have been made, for biological control services of predators and parasitoids to be more consistently included in pest management decision-making, there remains a need for simple metrics that are easily measured in the field and sufficiently robust to accurately predict the contributions of natural enemies to pest suppression through a field season. This ongoing challenge still needs to be met to raise the level of recognition of the significant role that predators and parasitoids play in both natural and managed ecosystems.

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Phytophagy

8

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8.1 Introduction

This chapter considers ways in which phytophagy by parasitoids and predators may be studied, particularly with respect to conservation biological control programmes that involve the manipulation of insect natural enemies by means of supplemental food provision. Many insect natural-enemy species, at least at some stage during their life-cycle, exploit plant-derived food in addition to insects (i.e., are omnivorous). They feed:

(1) Directly, upon plants, consuming floral and extrafloral nectar, pollen, seeds (either whole seeds or specific tissues), and, less commonly, materials such as plant sap (including the juices of fruits), epidermis, trichomes and plant tissue; and/or

(2) Indirectly, consuming honeydew (modified plant sap) produced by hemipterans, e.g., aphids, mealybugs, soft scale insects and whiteflies, among others, that feed on plant sap.

In many species of predatory insects, only the adults are phytophagous. This is the case, for example, in aphidophagous hoverflies, predatory ants and some lacewings where the adults feed exclusively on plant materials. In others, the larvae as well as the predatory adults feed on plants, e.g., the ladybird *Coccinella septempunctata*. Among parasitoids, generally only the adults consume plant-derived foods (part of the definition of the term 'parasitoid' is that the developing offspring feed exclusively on one host); exceptions include certain Eurytomidae which as larvae are zoophytophagous, developing initially as parasitoids and completing development by feeding upon plant tissues (Henneicke et al., 1992).

For many years, most investigations of predator or parasitoid foraging behaviour and population dynamics were concerned only with a natural enemy's interaction with its prey or host species, and either ignored or overlooked its interaction with non-prey/non-host food sources. However, since the 1990s there has been growing interest in the importance of plant-derived foods in the behaviour and ecology of parasitoids

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and predators (e.g., Evans, 1993; Jervis et al., 1993; Cisneros & Rosenheim, 1998; Gilbert & Jervis, 1998; Heimpel & Jervis, 2005; Wäckers et al., 2005, 2008; Bernstein & Jervis, 2008; Lundgren, 2009; Tena et al., 2016; Benelli et al., 2017; Gurr et al., 2017; Perovic et al., 2018; Heimpel, 2019).

Information on the range of prey or host types attacked by natural enemies remains far more comprehensive and detailed compared with information on the types of plant material that many consume. This difference in emphasis is to be expected, since researchers have tended to regard the consumption of plant materials as somewhat peripheral to what is generally considered to be the most important aspect of natural-enemy biology, namely entomophagy. Another reason is that the methods used to measure host/prey range in the field are far more developed than those used to determine the plant-derived sources of insect natural enemies, especially when the plant-derived food is honeydew.

In this chapter, we suggest how the source and identity of plant-derived food comprising the diet of natural enemies might be determined and discuss some ways in which this information might be used to gain key insights into the behaviour, physiology, population dynamics and pest control potential of parasitoids and predators. The chapter reflects our research bias: nectar-, pollen- and honeydew-feeding are emphasised over other types of feeding behaviour. For information on plant sap-feeding by predatory heteropteran bugs, see Naranjo Naranjo and Gibson (1996), Coll (1998), Eubanks and Strisky (2005), Castañé et al. (2011) and Pérez-Hedo et al. (2021), for information on seed-feeding by carabids and ants see Lundgren (2009) and Honek et al. (2003) and for information on plant feeding by predatory mites see van Rijn and Tanigoshi (1999), Nomikou et al. (2003), Adar et al. (2012), McMurtry et al. (2013) and Cruz-Miralles et al. (2019).

8.2 What Plant Materials Do Natural Enemies Feed upon and from What Sources?

8.2.1 Introduction

While it is well known that natural enemies feed on nectar, pollen and honeydew, the range of flowers and honeydews exploited as food under field conditions is poorly known for most species of predators and parasitoids. Knowing the food source used by natural enemies is especially important for biological control researchers when there are food sources with different accessibility and/or nutritional value. For example, in citrus, there are numerous species of hemipterans that excrete honeydew with different nutritional value for the natural enemies that feed on them (Tena et al., 2013a, 2013b, 2016). Similarly, in aphid-infested crops with floral resources, natural enemies have access to both nectar and honeydew with different accessibility and value for natural enemy fitness (e.g., Lee et al., 2006). Here, we review direct and indirect methods that researchers have used to ascertain the food sources used by natural enemies in the field.

8.2.2 Direct Observations on Insects

Floral Materials

Generally, insects, including even very small parasitoid species (e.g., most Chalcidoidea, Cynipoidea and Proctotrupoidea) can be easily observed visiting flowers and extrafloral nectar. Numerous direct observations of predators and parasitoids visiting flowers have been published over the past two centuries, many of these listed in works such as: Müller (1883), Willis and Burkill (1895), Knuth (1906, 1908, 1909) and Robertson's (1928) which deal with various flower-visiting insects; Drabble and Drabble (1927) with various Diptera; Allen (1929) with

Tachinidae in particular; Drabble and Drabble (1917) and Hamm (1934) with Syrphidae (hoverflies), including a few aphidophagous species. More recent records are in Parmenter (1956, 1961), Herting (1960), Karczewski (1967), Judd (1970), Kevan (1973), Sawoniewicz (1973), Barendregt (1975), Maier and Waldbauer (1979), Primack (1983), Toft (1983), Haslett (1989a), de Buck (1990), Maingay et al. (1991), Cowgill et al. (1993), Jervis et al. (1993), Al-Doghairi and Cranshaw (1999) and Colley and Luna (2000). Tooker and Hanks (2000) also analysed Robertson's (1928) data. Direct observations of natural enemies feeding at extrafloral nectaries have also been recorded (Nishida, 1958; Putman, 1963; Keeler, 1978; Beckmann & Stucky, 1981; Hespenheide, 1985; Bugg et al., 1987).

The mere presence of natural enemies on or in flowers does not in itself indicate that they are feeding from them. This and other key points should be considered when natural enemies are observed visiting flowers:

(1) Observers should record what materials (whether nectar, pollen or both) the insects are feeding upon, if they are feeding at all (natural enemies may visit flowers either accidentally or solely for purposes other than feeding, i.e., sheltering, meeting mates and locating prey and hosts, although it is likely that where flowers are visited for mating purposes, feeding also occurs (e.g., Toft, 1989a, 1989b; Belokobilskij & Jervis, 1998). If the insects can be seen (either with the naked eye or with magnification) to apply their mouthparts to a nectar or pollen source, then it is reasonable to infer that feeding is taking place. The same inference can be drawn from observations of parasitoids, ants and other natural enemies visiting extrafloral nectaries. In plants with concealed nectaries, it is more difficult to determine whether feeding is taking place and/or what materials are being fed upon, although in some cases it may be reasonably inferred from the insect's behaviour that nectar-feeding rather than pollen-feeding is taking place. For example, in the creeping buttercup, *Ranunculus repens*, and its close relatives, the nectaries are situated near the bases of the petals, and are each concealed by a flap or scale (Percival, 1965). The adults of a variety of small parasitoid wasps may be observed with their heads either at the flap edge or beneath the flap, suggesting nectar-feeding (Jervis et al., 1993). The activities of small parasitoids visiting some other flower types may be interpreted as nectar-seeking behaviour. For example, in the flowers of *Convolvulus repens and Calystegia sepium* (Convolvulaceae), wasps and ants crawl down the narrow passages at the base of the corolla that lead to the nectaries (see also Haber et al., 1981, on ants).

(2) With many plants that have concealed floral nectaries, particularly species with narrow corollas (e.g., members of the daisy family [Asteraceae]), it is sometimes possible to ascertain directly what materials the insect visitors are feeding on, although this is not the case with most parasitoid hymenopterans (Jervis et al., 1993). Even in the absence of close observations, it may be reasonable either to infer from the structure of the insects' mouthparts or from the insects' behaviour that particular plant materials are being sought. For example, among flies (nemestrinids, phasiine tachinids and some conopids) and among wasps (some chrysidids, leucospids and a few braconids and ichneumonids) some species have long, slender proboscides that are unlikely to be used for removing any materials other than floral nectar (Gilbert & Jervis, 1998; Jervis, 1998). Aphidophagous syrphids, however, use their proboscides for obtaining both pollen and nectar (Gilbert, 1981). Identification can sometimes be problematic for small parasitoids and this should be taken into consideration when reviewing the older literature on parasitoids; early publications probably contain a high proportion of misidentifications but parasitoid, especially wasp, taxonomy has since advanced greatly.

(3) When considering a particular natural enemy, one should not attach too much significance to those plant species for which no record of visits has been obtained. If the insect species being investigated has a preference for the flowers of certain plant species (Sect. 8.3), then the probability of the investigator recording that natural enemy on those species will be significantly greater than on others. Because of this, a superficial field survey could give the misleading impression that the least preferred plant species are not exploited at all. This is an important point to consider when dealing with almost all the published records of visits by insects to flowers.
(4) Finally, recording the sex of the insects involved greatly increases the scientific value of the information obtained.

Examination of plants in the field for natural enemies carrying out nectar- and pollen-feeding may prove extremely difficult and time-consuming (aphidophagous Syrphidae being an obvious exception). Flowers may therefore be presented to the insects in the laboratory, and observations on behaviour carried out. This has been done for many parasitoid species (e.g., Györfi, 1945; Leius, 1960; Shahjahan, 1974; Syme, 1975; Idris & Grafius, 1995; Patt et al., 1997; Drumtra & Stephen, 1999). However, the results of such tests need to be viewed with caution, because under field conditions the insects may not visit the same plant species as those with which they are presented in the laboratory. Even greater caution needs to be applied to the results of laboratory tests that involve presenting nectar extracted from different flowers to insects, as has been done for ants (Feinsinger & Swarm, 1978; Haber et al., 1981), because parasitoids might not be able to access the nectary under field conditions.

Honeydew

Hemipteran honeydew is hypothesised to be widely exploited as food by insect predators, including ants, and parasitoids in agroecosystems because honeydew is often the main sugar source available (see and Lundgren, 2009, for a broad-ranging review; Majerus, 1994, on Coccinellidae ; New, 2002, on Chrysopidae ; Gilbert & Jervis, 1998, and Jervis, 1998, on parasitoid flies; Tena et al., 2016, on hymenopteran parasitoids; Evans, 1993, on various natural enemies of aphids). The list of parasitoids and predators observed in the field feeding on, or searching for, honeydew is, however, much shorter than that for nectar-feeding. Honeydew feeding has received much less attention, mostly both because honeydew was considered a carbohydrate source of poorer quality compared to nectar (Wäckers et al., 2008) and because it is difficult to detect its presence in the field compared to finding nectar in flowers. Some parasitoids of honeydew-producing species take honeydew directly from the host's anus, as ants do (Rasekh et al., 2010). Honeydew can solidify very rapidly after deposition, becoming a crystalline sugar film, which many Diptera such as tachinids and syrphids can readily exploit using their fleshy labella (Allen, 1929; Downes & Dahlem, 1987; Gilbert & Jervis, 1998). Feeding on wet honeydew is likely to be practised by many parasitoid wasp species, particularly in arid habitats, but feeding on dried honeydew has been observed in only a few parasitoid wasp species (Bartlett, 1962).

Obtaining direct evidence of feeding by parasitoids on honeydew deposits can be problematical because: (1) the deposits can be difficult for the researcher to locate, despite being often highly abundant; (2) owing to their often great abundance within a habitat, a given-sized population of natural enemies is likely to be more highly dispersed over honeydew patches than over flowers; and (3) as mentioned above, some parasitoid wasps apply their mouthparts to honeydew films, but do so solely for the purpose of detecting host-finding kairomones (Budenberg, 1990). Finally, when several hemipteran species share the same patch in a plant (e.g., several aphid and psyllid species are generally observed in citrus flush) it might not be possible to determine the species that has excreted the honeydew from which the natural enemy is feeding.

Plant leachates

Plant leachates, which can contain high levels of carbohydrates, have been found on the surfaces of all plant species examined (Tukey, 1971). Sisterton and Averill (2002) observed the braconid wasp *Phanerotoma franklini* to apply its mouthparts to the surface of cranberry leaves in the field, a behaviour that suggested it was feeding on leachates. Biochemical analysis techniques (Sect. 8.2.3) would need to be used to confirm this. Given the ubiquity of leachates, and the knowledge that some non-parasitoid species feed on them (Stoffolano, 1995), their potential as a food source for natural enemies should be borne in mind when undertaking investigations of parasitoid feeding ecology (Sisterton & Averill, 2002).

Tukey (1971) considered plant guttation a category of leaching. Plant guttation is the fluid from xylem and phloem sap secreted at the margins and tips of leaves through hydathodes (Singh, 2016). Guttation drops generally contain low levels of carbohydrates and proteins and they have been considered a source of water for beneficial insects. However, Urbaneja-Bernat et al. (2020) found that guttation drops secreted by highbush blueberries contain high levels of carbohydrates, are present on leaves during the entire growing season, and are visited by numerous predators and parasitoids of different orders that increase their survival when feeding on it.

8.2.3 Indirect Methods

Where direct evidence of plant-derived food consumption is unavailable or when researchers are interested in quantifying the percentage of a population that is feeding on plant-derived sources, other evidence needs to be sought. Here we consider indirect methods for documenting and quantifying consumption of various plant-derived food sources by natural enemies.

Pollen

The body surface, including the mouthparts, of flower-visitors may be examined for the presence of pollen grains irrespective of whether the insects are collected at flowers (e.g., Holloway, 1976; Stelleman & Meeuse, 1976; Gilbert, 1981; Dafni, 1992) or in other circumstances. The plant species source of such grains can be identified either: (1) by using identification works (e.g., Reitsma, 1966; Erdtman, 1969; Sawyer, 1981, 1988; Faegri & Iversen, 1989; Moore et al., 1991); and/or (2) by comparing the grains with those collected by the investigator from plants in the parasitoid's field habitat (flowering plant species, in some cases even closely related ones, differ with respect to pollen surface sculpturing).

However, the presence of pollen grains on the body surface, even on the mouthparts, does not necessarily constitute proof that pollen is ingested, since the insects may become contaminated with grains whilst seeking nectar. For example, Lysenkov and Galinskaya (2017) did not find a correlation between the pollen compositions in the guts and on the bodies of three genera of hoverflies. When collecting insects for the purpose of examining their body surface for pollen grains, it is essential that insects are individually isolated to prevent cross-contamination with (sticky type) grains. The body surface of insects can also be examined for the presence of fungal spores, which insects may passively accumulate as they brush against fruiting bodies. If spores are present, it does not necessarily indicate that the insects feed on fungal materials, although some parasitoids do feed on the sugar-rich spermatial fluid of fungi (Rathay, 1883).

Gut dissections may reveal the presence of pollen grains. This technique has been used with hoverflies (e.g., Holloway, 1976; Leereveld, 1982; Haslett, 1989a; Hickman et al., 2001; Villa et al., 2016), parasitoid wasps (Györfi, 1945; Leius, 1963; Hocking, 1967; Jervis et al., 1993), coccinellid beetles (Hemptinne & Desprets, 1986; Hoogendoorn & Heimpel, 2004; Lundgren et al., 2004; Ricci et al., 2005), and green lacewings (Sheldon & MacLeod, 1971; Villaneve et al., 2005). This method has been applied to immature and adult specimens that were dried, preserved in ethanol, and deep-frozen (Holloway, 1976; Leereveld, 1982; Haslett, 1989a). Because the exines (outer coverings) of pollen grains are

refractory structures (i.e., they are resistant to either decay or chemical treatment), they retain much of their original structure (hoverflies, at least, do not have to grind pollen in order to extract nutrients; Gilbert, 1981; Haslett, 1983), and the original plant source can thus often be identified, as indicated above. Hunt et al. (1991) devised a pollen exine detection method in which the abdomens (or gasters in the case of wasps) of dried, preserved insects are cleansed, crushed, heated in a mixture of acetic anhydride and concentrated sulphuric acid, the mixture centrifuged, and the pollen exines subsequently isolated and identified as above (for further details, see Lewis et al., 1983; Hunt et al., 1991).

Golding and Edmunds (2003) proposed and tested the use of frass pellets of different species of aphidophagous lady beetles to detect the presence of pollen. Beetles were held for 48 h in the laboratory and the largest faecal pellet produced by each individual during this 48-h holding period was selected for dissection and analysis. However, since so many types of pollen and fungal spores could be found in faecal pellets, they combined and scored them as a single category.

Electrophoresis of the gut contents or similar methods (Sect. 6.2.9) also have potential as a method for detecting the presence of pollen in the diets of arthropods and can be particularly useful for the identification of pollen to the species level (van der Geest & Overmeer, 1985; Corey et al., 1998). Indeed, the presence of pollen in hoverflies, as well as other natural enemies, can be identified using DNA metabarcoding (Lucas et al., 2018). This technique does not require a high level of experience in the taxonomic identification of pollen.

The presence of pollen exines within the gut or faeces of a predator or parasitoid does not necessarily indicate that the insect has been feeding directly at pollen sources (i.e., anthers). Pollen may fall or be blown from anthers and subsequently become trapped in nectar, honeydew or dew (Todd & Vansell, 1942; Townes, 1958; Hassan, 1967; Sheldon & MacLeod, 1971). It is possible that, for some species, the consumption of nectar, honeydew or dew is the sole means of obtaining pollen. Also, with predators such as carabid beetles, pollen grains may enter the gut via the prey (Dawson, 1965). Lastly, pollen grains that land upon the surfaces of leaves are deliberately taken by some insects, e.g., adults of the non-aphidophagous hoverfly genus *Xylota* (Gilbert, 1991), and it is possible that some parasitoid flies do the same (Gilbert & Jervis, 1998).

Orb-weaving spiders (Araneidae) are commonly regarded as generalist predators but their webs can also trap pollen. Eggs and Sanders (2013) found, using stable isotope ratios, that pollen was a substantial component of the diet of two species of orb-weaving spiders. They suggested that pollen ingestion was not accidental because the pollen grains in their study were rather large and had first to be digested extra-orally by enzymes in an active act of consumption.

Sugars (nectar and honeydew)

Plant-derived sugars in the guts of parasitoids and predators can be detected using various biochemical techniques that were originally designed for biting (mammal-blood feeding) flies of various kinds (Jervis et al., 1992; Heimpel et al., 2004; Heimpel & Jervis, 2005; Lee, 2019). Van Handel (1972, 1984) developed a series of simple biochemical colorimetric assays based on anthrone (9(10*H*)-Anthracenone; $C_{14}H_{10}O$). Solutions of anthrone and sulphuric acid change from yellow to blue-green upon contact with most sugars (Morris, 1948; Seifter et al., 1950; Scott & Melvin, 1953), and the specific sugars that induce the colour change vary with temperature. Fructose (either by itself or as a moeity of other sugars including sucrose) reacts with anthrone at room temperature within an hour (the 'cold anthrone test'), whereas various other sugars require short incubation at 90ºC or a much longer incubation of at least 12 h at room temperature. Given the absence of fructose and sucrose from insect haemolymph, a positive reaction from an insect by means of the cold anthrone test signifies the presence of either or both of these plant-derived sugars in the insect's gut. The cold anthrone test has since been used on laboratory and field-caught parasitoids (e.g., Olson et al., 2000; Fadamiro & Heimpel, 2001;

Lee & Heimpel, 2003; Lee et al., 2004; Fadamiro & Chen, 2005; Heimpel & Jervis, 2005; Lee et al., 2006; Wyckhuys et al., 2008; Rand & Lundgren, 2018; Tena et al., 2018). The incidence of anthrone test-positive individuals in field populations of parasitoids ranges considerably, from 20% for *Macrocentrus grandii*, *Trichogramma ostriniae* and *Aphytis aonidiae* to > 70% for *Cotesia glomerata* and *C. rubecula*. This method has been also used for predators such as coccinelids (Lundgren & Seagraves, 2011; Seagraves et al., 2011). Heimpel et al. (2004) developed a cold anthrone technique for small-bodied parasitoid wasps, involving the placing of individuals into a small droplet of anthrone on a microscope slide, squashing the insect with a cover slip, and recording the presence (sugar positive) or absence (negative) of a green halo around the parasitoid's body. This method was later used by Segoli and Rosenheim (1998) and Kishinevsky et al. (2018) to evaluate a large number of parasitoids, identifying those that had recently fed on carbohydrates and others that had not fed.

The cold anthrone test cannot distinguish between sugars from nectar (floral or extrafloral), honeydew, or other much less common sources such as plant exudates, fruit juices or artificial sugar sprays. Chromatographic techniques (HPLC–high performance liquid chromatography, GC–gas chromatography, and TLC–thin layer chromatography) can tell us much more about the specific kinds of sugar present in insect guts. Honeydews have signature sugars (most notably erlose, melezitose, trehalulose and stachyose, Zoebelein, 1956; Wäckers, 2001; Wyckhuys et al., 2008; Tena et al., 2013b), whereas nectars generally do not. These signature sugars can be used to determine honeydew feeding by natural enemies. However, precaution should be taken because some parasitoids are able to synthesise these sugars (Wäckers et al., 2006a, 2006b). Even in these cases, however, the proportion of signature sugars can be used to determine honeydew feeding (Hogervorst et al., 2007; Tena et al., 2013b; Calabuig et al., 2015). It can be more difficult to determine the hemipteran species that has excreted the honeydew on which the natural enemy has fed when several hemipteran coexist in the field (Tena et al., 2016). The melezitose component of the honeydew produced by some aphid species can vary based upon the plant species the aphids have fed upon (Fischer et al., 2005) and can also be influenced by ant tending (Fischer & Shingleton, 2001).

A new enzymatic method for measuring insect sugar concentrations has been developed that could be used to determine nectar-feeding in natural enemies (Phillips et al., 2018). This method has been tested for use in measuring glucose, fructose and trehalose in the parasitoids *Microctonus aethiopoides* and *M. hyperodae* under controlled conditions. Compared to the previous tests (cold anthrone and HPLC), this enzymatic method is quicker and less expensive than HPLC, and is safer, faster and more sensitive than the anthrone test. If the method allows the measurement of other sugars, it could in principle also be used to investigate honeydew feeding.

Another indirect method of determining whether natural enemies consume sugar-rich foods is to mark potential food sources with dyes or other markers such as rare elements (e.g., rubidium) or radioactive elements (e.g., $H_3{}^{32}PO_4$), and examine the guts of the parasitoids or predators for the presence of the marker (e.g., Freeman-Long et al., 1998). However, it is important to be confident that the label truly has been obtained via the presumed food type: it might mark pollen or it could be passed onto herbivores feeding on the labelled plants and subsequently onto predators and parasitoids feeding on these herbivores (Payne & Wood, 1984; Jackson et al., 1988; Hopper, 1991; Hopper & Woolson, 1991; Jackson, 1991; Corbett & Rosenheim, 1996). Acquisition of the marker via pollen can be ruled out if gut dissections do not reveal the presence of pollen exines in any individuals from among a reasonable-sized sample. A simpler method could be colouring the food source, as has been proposed by Joschinski and Krauss (2017) for aphid honeydew. The problem of using markers is to ensure adequate uptake of the marker by the plants, and it may be necessary to apply the marker to the nectar of a large number of flower species and to a large number of honeydew

production sites. Furthermore, it is vital to ensure that the marker is not repellent or toxic. Finally, several studies have employed a combined NMR and liquid chromatography–mass spectrometry (LC–MS) metabolomics approach to simultaneously assess differences in individual lipids and complementary information on polar metabolite concentrations, including haemolymph sugars, amino acids, and organic acids (Kapranas et al., 2016; Snart et al., 2018). This approach was used to compare the nutritional profile of wasps with different diets and ages under controlled conditions and could also be used to identify plant–insect interactions in the field (Snart et al., 2015). A common problem of all of these methods is that, for natural enemies that feed on nectar and honeydew but also on plant sap, it may be difficult to determine whether they have fed on plant-derived materials or sap. These include mirid bugs and phytoseiid mites.

Seed predation by ants, crickets, some carabids and other insects can be important in limiting the spread of weedy species (e.g., Lundgren, 2009). Seed predation by ants in particular can be established by examining the 'booty' being carried in ant columns (Sect. 6.3.3). The columns can also be followed to determine which plant species are being visited, although an important point to bear in mind is that the ants may be taking seeds from the ground instead of from the plants themselves.

8.3 Do Natural Enemies Show Specificity in the Plant Food Sources They Exploit?

Only in a relatively small number of cases can we be confident that a predator or parasitoid species is discriminating in the food sources it visits under field conditions. Nevertheless, we can reasonably expect some degree of behavioural specificity (i.e., specificity beyond that attributable to spatio-temporal synchrony between insects and food sources) to be shown generally among natural enemies.

The range of different food types exploited will depend on insect morphology to some extent. In tachinid flies, possession of a greatly elongated proboscis is linked to exploitation of floral nectar only, while a moderately long or a short proboscis is linked to feeding on a broader range of sugar-rich food types (floral nectar, honeydew, extrafloral nectaries) (Gilbert & Jervis, 1998, using field observation data of Allen, 1929). Among flower-visitors, the range of plant species exploited will depend largely also on flower anatomy, reflecting a correlation between proboscis length and diet (van Rijn & Wäckers, 2016). Within the Syrphidae (hoverflies), as proboscis length increases, the flies are more often found to be associated with flowers having deep corollae, and the proportion of nectar in the diet increases (pollen being the alternative food, see below) (Gilbert, 1981). In the case of *Episyrphus balteatus* (the 'marmalade hoverfly'), feeding is related to effective flower depth and the critical flower depth is 1.6 mm, which is less than the proboscis size of this hoverfly. The critical floret depth, however, may vary among plant species and it is less than 1.0 mm for Asteraceae (van Rijn & Wäckers, 2016). In parasitoid wasps also, proboscis type and length appear to be correlated with the degree of nectar concealment (Gilbert & Jervis, 1998; Jervis, 1998). Corbet (2000) has shown that, in butterflies, body mass and wing loading, in addition to proboscis length, determine which flower type is visited (flower type being defined in terms of corolla depth and the degree of flower clustering); the same may apply to predators and parasitoids.

Among flower-visitors, the range of plant species exploited will also depend upon colour and odour (Shahjahan, 1974; Gilbert, 1981; Haslett, 1989b; Jervis et al., 1993; Wäckers, 1994; Idris & Grafius, 1995; Orr & Pleasants, 1996; Wäckers et al., 1996; Baggen et al., 1999; Patt et al., 1999; Sutherland et al., 1999; Wäckers & van Rijn, 2012; Géneau et al., 2013; Foti et al., 2017). Taste and nectar toxicity undoubtedly also play a role (Wäckers, 1999; Gardener & Gillman, 2002). Microorganisms such as bacteria can affect nectar chemistry by altering its acidity, sugar and amino acid composition/concentration and by adding synthesised compounds. Lenaerts et al. (2017) changed the composition of nectar by

adding different groups of bacteria: these inoculations did not affect nectar consumption but did affect the longevity of parasitoids that fed on the nectar.

In parasitoids, the compatibility of morphology (body size, proboscis length) with flower anatomy can sometimes be easily inferred from size measurements (Jervis et al., 1993), although observations of parasitoid behaviour can provide more useful insights into constraints imposed by floral anatomy (e.g., Plepys et al., 2002). When conducting such assays, the nutritional status of the parasitoid and the stage of the plant should be taken into consideration because flower odours and the response of parasitoids might change with the stage of the plant and the status of parasitoid (Takasu & Lewis, 1993; Jönsson et al., 2005; Bianchi & Wäckers, 2008). Moreover, as in the case with host searching, the selectivity of parasitoids can also change with their previous experience. Thus, parasitoids can become attracted by a flower after feeding on its nectar (Takasu & Lewis, 1993; Wäckers et al., 2006a, 2006b; Fataar et al., 2019).

Selectivity by hoverflies (Gilbert, 1981; Haslett, 1989a; Cowgill et al., 1993; Inouye et al., 2015), and also by bee-flies (Toft, 1983), varies among species, and the same undoubtedly applies to parasitoids. Some species exploit the flowers of only a few plant species, in some cases only one (Inouye et al., 2015). Toft (1983), for example, found several bee-fly species to be restricted to the flowers of one plant species. At the other extreme are species such as the aphidophagous hoverfly *Episyrphus balteatus*, which exploits a much larger number of plant species. The terms 'specialist' and 'generalist' are used to distinguish between the two types (Toft, 1983; Haslett, 1989a). Generalist flower-visitors exploiting the flowers of a range of concurrently blooming plant species are very likely to behave as butterflies and some other insects do, visiting some flower types more frequently than would be expected on the basis of their respective abundances, thereby displaying a preference (defined in Sect. 1.6.7). Preferences are unlikely to be fixed and might change:

(1) As individual insects respond to the changing profitability of any of the plant species within its food plant range, for example due to: (1) exploitation by competitors for the resource (as shown by Toft, 1984, for bombyliid flies; Sect. 8.3); (2) phenological changes in flower abundance and dispersion through the season; (3) changes in nectar secretion rates through the day; and/or;
(2) As the nutritional requirements of the insects themselves change, either through their lifetimes or through the day. Adult syrphids display flower constancy in the manner of bees (Gilbert & Owen, 1990; Goulson & Wright, 1998; Goulson, 2000). That is, individuals specialise temporarily (e.g., for the duration of a foraging bout, or over several successive bouts) on one flower species. Flower constancy in syrphids is due to a combination of preferences for flower height, colour, and type preference in combination with local flowering phenology (Ssymank, 2003, in Inouye et al., 2015). Protocols for the measurement of flower constancy are given by Dafni (1992) and Goulson and Wright (1998). For a discussion of the adaptive significance of flower constancy, see Dafni (1992), Goulson (2000) and Inouye et al. (2015).

In flower-visiting nectarivores, food plant ranges and preferences are likely to be based upon the following floral characteristics that could affect the insect's foraging energetics: flower abundance and dispersion, nectar volume, concentration and degree of accessibility. Since nectar is a source not only of sugars (energy) but also of other nutrient classes (amino acids occur in a wide range of nectars, Baker and Baker, 1983), a preference could also be based upon the net rate of acquisition of these metabolites. The relationship between attractiveness of flowers to parasitoids and enhancement of parasitoid fitness has received little attention (for exceptions see Géneau et al., 2013; Foti et al., 2017). The

preference could have a more complex basis, such as the relationship between nectar sugar concentration and amino acid concentration. Toxins or the composition of microorganisms in the nectar could also play a role (Adler, 2000; Lenaerts et al., 2017). Clearly, any investigation that attempts to relate flower preference to the above-mentioned floral characteristics could prove very complex if combined nectar and pollen-feeding by flower-visitors is considered.

The standard method of assessing (i.e., detecting and measuring) flower preferences in insects in the field is to record the relative frequency with which an insect species visits the flowers of each of a range of available plant species measured in terms of the number of sightings of individual foragers during a census walk (Sect. 6.2.6), and relating this to the abundances of the different flower types (Toft, 1983, 1984; Cowgill et al., 1993). The number and species of natural enemies present per flower can be also measured by collecting the flowers in bags and extracting the arthropods in the laboratory (Wäckers & van Rijn, 2012 give more details). As an example of these studies, a 50 $\times$ 1 m sampling area containing a range of flowering plants was traversed at a constant speed on each observation day, and all sightings of the hoverfly *Episyrphus balteatus* were recorded (Cowgill et al., 1993). The behaviour of the insects at the time of first sighting was noted. Data gathered in this way can be analysed to determine whether or not the different flower species are visited in proportion to their abundances (i.e., detection of preference; Sect. 1.6.7) and to determine to what degree each species is preferred.

In such studies, the following challenges need to be addressed:

(1) Can the flowers of different plant species, particularly those of different structural types, be considered as equivalent foraging units? Observations of the insect's foraging behaviour upon particular flower types, made prior to the preference study, could be useful in resolving this question; such an approach is better than making assumptions as to what the insect perceives as a patch (Chap. 1).

(2) Can the flowers of different plant species be considered as equivalent resource units? Published studies on flower preferences of hoverflies have considered preferences in terms of floral abundance and visitation rates, instead of more realistically in terms of the availability and quality of food materials and the consumption rates of the insects. Plant species may differ both in the rate at which they produce pollen or nectar, and in their corresponding biochemical content. Also, for the forager there may be differences in handling times among plant species (Haslett, 1989a). Handling time (defined in Sect. 1.14) will depend upon the rate of nectar secretion and on nectar viscosity (Harder, 1983).

A number of interacting variables may thus be involved in flower preference, making an objective assessment of the factors determining flower selectivity difficult, especially if the insects utilise both pollen and nectar (Haslett, 1989a).

To overcome the drawbacks associated with methods involving behavioural observation, Haslett (1989a) examined the gut contents of hoverflies in his study of flower selectivity, counting the number of pollen grains found and identifying their source. He measured pollen availability in terms of a 'floral area' index, which was calculated partly on the basis of flower diameter, an index that is unlikely to provide a realistic measure of pollen availability, thus compromising the value of his preference analyses. Toft (1983), however, argued that corolla diameter can be used as a reasonable relative measure of the nectar content of different flower types, an assertion that still requires verification. Toft (1983) expressed resource availability in terms of flower number, both unweighted and weighted by corolla diameter, and found that weighting altered preference estimates only slightly. Nowadays, pollen identification can be simplified and facilitated using DNA metabarcoding (Lucas et al., 2018).

While the range of flowers used by natural enemies, mainly hoverflies, has been studied, their preference for different honeydews needs to be understood as well. It is expected that natural enemies of honeydew producers feed on the honeydew excreted by their host or prey species. This assumption has never been tested, but Lenaerts et al. (2017) have demonstrated that the aphid parasitoid *Aphidius ervi* prefers and consumes more sugars that occur in honeydew (i.e., sucrose, fructose or melezitose) than those that do not (glucose and rhamnose). This situation is different for natural enemies of non-honeydew producers, which have to search in different patches for carbohydrate sources and for hosts or prey. In some ecosystems, these natural enemies can select among different honeydews during their search for carbohydrates. For example, many hemipteran species feed on citrus and excrete honeydew with different nutritional values for parasitoids of non-honeydew producers (Tena et al., 2013a, 2013b). The parasitoid *Aphytis melinus* increases its longevity up to ten days when it feeds on honeydew excreted by the mealybug *Planococcus citri* but not by the whitefly *Aleurothrixus floccosus.* A similar phenomenon occurs in olive orchards, where the black scale *Saissetia oleae* and the olive psyllid *Euphyllura olivina* (both in the order Hemiptera) each excrete honeydew of different nutritional value in spring and in the summer (Villa et al., 2016). Whether natural enemies show a preference for any of these honeydews remains to be investigated.

Studies on parasitoids and predators that combine realistic measurements of floral food availability and consumption have yet to be carried out. Hoverflies are perhaps not ideal subjects for such research, since they consume both pollen and nectar, while parasitoids, which rarely feed directly upon pollen (Jervis et al., 1993; Gilbert & Jervis, 1998; Jervis, 1998), are less easy to observe in the field. Nevertheless, there is a pressing need for information on the flower preferences of natural enemies, due to an increasing awareness among biological control researchers of the potential importance of non-prey and non-host foods in the population dynamics of natural enemies (Powell, 1986; Kidd & Jervis, 1989; Jervis & Kidd, 1999; Heimpel & Jervis, 2005; Heimpel, 2019; Lee, 2019) (Sect. 8.6) and the need to establish the role of natural enemies as pollinators (Sect. 8.7).

8.4 Interpreting Patterns of Resource Utilisation

Coexisting hoverfly and bee-fly species divide up the available floral resources in the following (not necessarily exclusive) ways: (1) by being active at different times of the day (Toft, 1984; Gilbert, 1985a; D'Amen et al., 2013; Inouye et al., 2015); (2) by exploiting a different range of flower species (Gilbert, 1980, 1981; Toft, 1983; Haslett, 1989a; Ionuye et al., 2015); and (3) by exploiting nectar and pollen in different proportions (Gilbert, 1981, 1985b).

Are species differences in resource exploitation the result of competition for limited resources? Gilbert (1985a) investigated the diurnal activity patterns of several hoverfly species in the field. He carried out census walks during which he noted what types of behaviour individual flies were performing and used the data to construct activity budgets for each species. With each observation of a fly, he measured ambient light intensity, temperature and humidity. From his observations and measurements, Gilbert (1985a) concluded that the differences in diurnal activity patterns are partly due to: thermal constraints (flies need to maintain a 'thermal balance'; Willmer (1983) discusses this concept, and Unwin and Corbet (1991) give details of techniques involved in the measurement of microclimate), and the need to synchronise their visits with the pollen and nectar production times of flowers. He did not invoke interspecific competition as a possible cause of the observed patterns of activity.

Toft (1984) constructed diurnal activity budgets for two coexisting species of bombyliids (*Lordotus*) that feed almost exclusively upon the flowers of *Chrysothamnus nauseosus* (Asteraceae). She found that one species, *L. pulchrissimus*, engaged in aggressive interactions, and

visited flowers mainly in the morning, while *L. miscellus* performed these activities over a much longer period of the day. Toft (1984) argued that competition is the cause of the interspecific differences recorded, concluding that these results corroborate the conclusion drawn in her study on bee-fly communities (Toft, 1983, 1984).

As for floral food selectivity, several investigations aimed at testing whether competition is responsible for patterns of resource partitioning among taxonomic groups of flower-visitors have been carried out, but few have dealt with a group of insect natural enemies. Toft (1983, 1984) adopted an observational approach by assessing preferences for flower species and measuring niche breadths at two study sites, concluding that patterns of resource utilisation by bee-flies resulted from interspecific competition. Interference competition was thought not to be a significant mechanism due to a lack of convincing evidence for interspecific aggression. Exploitation competition was thought more likely on the evidence that members of two genera (*Pthiria* and *Oligodranes*) tend to have longer mouthparts than a third (*Geron*), suggesting they may reduce nectar levels in flower corollas to such a level that they cannot be exploited by *Geron*.

Gilbert and Owen (1990) used another observational approach to investigate determinants of resource partitioning among hoverflies: they looked for evidence of interspecific competition as indicated by population fluctuations and morphological relationships between species. They tested the hypothesis that competition will be stronger between species belonging to the same guild (i.e., species having similar ecological requirements) and that morphologically similar species will compete more strongly. If this hypothesis is correct, strong interspecific competition ought to be evident as reciprocal fluctuations in density among the species. Gilbert (1985b) had already established that morphological similarity (measured in terms of distance apart in multivariate space) and ecological similarity (foraging niche overlap) are correlated: species with similar size and shape feed on similar types of flowers and take similar floral food types. Gilbert and Owen (1990) found no convincing evidence that members of the same adult feeding guild compete: reciprocal fluctuations in population density did not occur, and thus species appeared to be 'tracking' resources independently of one another.

An alternative to the above observational approaches to studying competition for food resources among parasitoids and predators would be to manipulate the insect populations, for instance, by removing individuals of one or more species and seeking evidence of competitive release in the remaining members of the community. Inouye (1978) and Bowers (1985) have shown that this approach can be successfully applied to bumblebees. Gilbert and Owen (1990), however, expressed the view that manipulation experiments on hoverflies represent an unrealistic goal because of the great mobility of adult flies, and suggested a way of circumventing the problem, at least in the case of woodland species (F.S. Gilbert, personal communication): manipulating species densities by continually adding laboratory-reared hoverflies to, rather than by removing flies from, a site.

The daily pattern of the utilisation of carbohydrate resources has also been studied in several species of parasitoids by measuring the amounts of sugars present with HPLC (Tena et al., 2013b; Dieckhoff et al., 2014) or by determining the presence of fructose with cold anthrone (Segoli & Rosenheim, 2013). The percentage of mymarids of the genus *Anagrus* feeding on sugars tended to increase throughout the day and feeding did not occur at night (Segoli & Rosenheim, 2013). Segoli and Rosenheim (2013) suggested that floral nectar was likely to be the main sugar resource for these mymarids. In contrast, the percentage of braconid *Binodoxys communis* and the aphelinid *Aphytis melinus* that had recently fed on sugar sources tended to decrease. Both species of parasitoids feed commonly on honeydew. Therefore, this pattern suggests that they feed on honeydew either at the end or the beginning of the day (Tena et al., 2013b; Dieckhoff et al., 2014). Competition with ants can also affect the pattern of honeydew utilisation as a sugar source: Calabuig et al. (2015) demonstrated that the sugar-feeding incidence of

both the parasitoid *Aphytis chrysomphali* and the predator *Chrysoperla carnea* on citrus trees in summer was negatively affected by the activity of the ants *Lasius grandis*, *Pheidole pallidula* and *Plagiolepis schmitzii.*

8.5 Insect Preferences and Foraging Energetics

The relationship of insect predators and parasitoids to plant-derived foods has hardly been considered from the point of view of foraging models (for discussions of general models, see Pyke, 1983, Stephens & Krebs, 1986, and Pleasants, 1989, and for parasitoid–host models see Sirot & Bernstein, 1996, and Tenhumberg et al., 2006). We are concerned here with the characteristics of nectar sources (extrafloral nectaries, as well as flowers) and their insect visitors, that may need to be quantified in experimental or observational tests of foraging models.

As far as nectar-feeding is concerned, the literature on butterflies (e.g., Boggs, 1987; May, 1988; Corbet, 2000) and bees (Heinrich, 1975; Hodges, 1985a, 1985b; Waddington, 1987; Pleasants, 1989; Corbet et al., 1995; Cresswell et al., 2000) provides useful information on approaches and techniques to adopt when planning investigations into the foraging behaviour, including flower preferences, of natural enemies (see also the textbooks of Dafni, 1992, and Kearns & Inouye, 1995). Such investigations, if carried out on nectarivores, may involve quantifying, or simply recording, some or all the following characteristics of the nectar sources.

A: The mean energy content of individual flowers or florets of different flower types. Energy content is determined primarily by volume and sugar concentration. The latter variables need to be measured during the periods of the day when the insects feed most frequently. For information on methodologies relating to the sampling of nectar, measurement of nectar volume and concentration, and the chemical analysis of nectar constituents, see Corbet (1978), Bolten et al. (1979), Corbet et al. (1979), McKenna and Thomson (1988), May (1988), Dafni (1992) and Kearns and Inouye (1995). The concentration of nectars that are mixtures of glucose, fructose and sucrose can be expressed as the equivalent amount of sucrose. Given the concentration and volume of the nectar, milligrammes of sugar per flower or per floret can be calculated and this quantity converted to energy per flower or per floret, assuming 16.8 J/mg of sucrose (Dafni, 1992). Note that care must be exercised when converting from refractometer readings of nectar concentration to milligrams of sucrose equivalents (Bolten et al., 1979).

In studies of nectarivory, nectar has either been sampled from flowers or florets that have been protected against visits or it has been taken from unprotected flowers or florets. With the former method, problems of interpretation can arise because the nectar can: (1) accumulate to abnormally high levels when flowers are protected (Lee & Heimpel, 2003); (2) decline in quantity due to nectar resorption by the plant (Burquez & Corbet, 1991); and (3) remain at around the original level (in some plant species removal of nectar increases net production of nectar, Pyke, 1991).

Thus, we recommend that data be obtained from unprotected flowers only. Of course, diel variation in nectar availability and concentration also ought to be measured (for further discussion of the practical aspects of nectar sampling and analysis, see Dafni, 1992, Kearns & Inouye, 1995, and Lee & Heimpel, 2003).

B: The relative accessibility of nectar in different flower types, e.g., distance from corolla opening to the nectar. Insects lacking an elongated proboscis, when extracting nectar from flowers or florets with long, tubular corollas, are likely to incur a larger handling cost, in terms of both time and energy, than when extracting nectar from flowers with either short corollas or completely exposed nectaries (see also **C**).

Some parasitoids have an elongated proboscis (modified labiomaxillary complex, Gilbert and Jervis (1998) and Jervis (1998)). Using artificial flowers (capillary tubes), Harder (1983) showed that in bees the advantage of such an elongated

proboscis depends on body size: large-bodied, long-tongued species (the tongue being the key functional part of the proboscis mouthparts in terms of nectar extraction) ingested nectar (sugar solution of fixed concentration) more rapidly than short-tongued species of equivalent size. Small-bodied, long-tongued bees ingested it at the same rate as small-bodied short-tongued bees. Note, however, that a long tongue can constitute a handling time constraint when a series of corollas is exploited on a flower (Plowright & Plowright, 1997).

C: The dispersion pattern of flowers or florets in each plant species. The degree of clumping of these plant parts will have an important bearing upon the insect's foraging behaviour, determining the amount of time spent travelling between the parts and the number of parts visited per unit of time (Corbet, 2000).

D: The abundances (density per unit area) of the different flower types. In one example of floral density assessment, Baldock et al. (2015) sampled flowers at intervals along transects in different ecosystems. All flowering plant species in a defined quadrat were identified and counted for each species.

The following characteristics of the insect's foraging behaviour, in relation to certain flower types, may need to be quantified:

E: The number of inflorescences, flowers or florets visited per foraging bout, and the number of foraging bouts performed per observation period. These features allow the flower visitation rate (number of inflorescences, flowers or florets visited per unit time) to be calculated (see Goulson, 2000, for a protocol). A 'bout' may be difficult to define. May (1988) took it to be the time period between a butterfly's entrance and exit either from a flower patch (it is unclear whether he meant an inflorescence or a group of inflorescences) or from his field of view. The number of nectar sources probed per unit time will depend on the time spent travelling between them (**F**, below) and on handling time (**G**, below).

Goulson (2000) addressed the question of why empirical studies have shown flower-visitors to visit a larger number, but a smaller proportion, of flowers in larger patches compared with smaller patches. He concluded that this behaviour is an optimal strategy, as searching for the remaining unvisited inflorescences is easier in a small patch. Goulson (2000) also showed that a departure rule, based on two successive encounters with empty inflorescences, closely predicts the observed behaviour.

F: The mean time spent travelling (either walking or flying) between flower patches, between inflorescences, flowers or florets of a particular plant species during a foraging bout. A protocol for measuring between-patch time allocation can be found in Goulson (2000). It is important to observe the foraging behaviour of individual insects closely, as this will reveal, for example, whether the insects fly between flowers but walk between the florets comprising an individual flower (this has important implications in terms of energy expenditure), and whether one or a series of flowers is visited during a foraging bout.

The foraging movements of a non-aphidophagous hoverfly (*Eristalis tenax*) upon flower capitulae of *Aster novae-angliae* (family Asteraceae) were elucidated by Gilbert (1983). The flies systematically probe the nectar-producing disc florets which form a narrow ring, leaving the capitulum when they have circled it once. They probe a variable number of florets on each capitulum, a behaviour that is thought to result from decisions made by the insect after each floret is visited about whether to probe another floret on the same capitulum or move to another capitulum (Pyke, 1984; Hodges, 1985a, 1985b). The 'rule' nectarivores use in making these decisions may be a threshold departure rule, i.e., if the reward (nectar) received from the current floret is less than a certain threshold amount, then the insect should leave the capitulum and locate a different one. Pleasants (1989) provides a discussion of how one might test whether: (1) insects that probe a variable number of florets per capitulum use a threshold departure rule to decide whether to continue foraging on a capitulum or to leave it

and locate another one (e.g., hoverflies, see above), or (2) the threshold used is optimal, i.e., maximises the energy intake rate.

G: The mean time spent dealing with each flower or floret of a flower type (probing it, perching on it, climbing into it, feeding, climbing out of it), i.e., **the handling time**. See **B**, above. This will depend in part on 'tongue' length, corolla depth and on the nectar volume per flower/floret.

H: The gross energy intake during foraging of the insect for a given flower type. This can be calculated, either on a per-bout or per-observation period basis, as follows:

$$E_{in} = R_v E_x \tag{8.1}$$

where E_{in} is energy intake, R_v is number of flowers visited per bout, and E_x is the mean quantity of energy (joules) extracted per flower/floret as measured in unprotected flowers (see above) at the same time of day. The gross energy intake per second can be calculated by dividing by the mean length, in seconds, of the foraging bouts.

For insects that are able to consume relatively large amounts of nectar during a foraging bout, the volume of nectar removed from each flower or floret can be measured and the mean quantity of energy extracted per flower or floret calculated by multiplying this amount by the nectar concentration, with suitable corrections (Bolten et al., 1979). The amount of nectar removed can be measured perhaps most easily either by comparing the nectar volume in flowers or florets visited once by an individual insect with the volume in non-visited flowers or florets or any weight gain in the insect immediately after it has finished feeding at the flower or floret. Note that the quantity of nectar extracted may alter with any changes in nectar concentration that may occur, and may also alter as successive flowers/florets are visited, due to satiation or gut limitation. The mean quantity of energy extracted per flower may also vary with factors such as flower or floret density, the amount of nectar removed per flower decreasing with increasing flower or floret (and therefore nectar) availability (Heinrich, 1975). Average and maximum crop volumes can also be measured quite easily in insects such as hoverflies (Gilbert, 1983).

Insects such as small parasitoids will remove very small amounts of nectar from a flower or floret, so estimating the volume of nectar extracted by comparing visited and non-visited flowers is likely to be difficult. Therefore, measuring either crop volume or weight gain in insects that have recently fed may be better alternatives. Again, measurements need to be taken at several stages during a foraging bout.

I: The energy expenditure of insects in relation to a given flower type. Following May (1988), this can be calculated, on a per-bout or per-observation period basis, as follows:

$$E_{cost} = \left(T_{fl}E_{fl}\right) + \left(T_h E_h\right) \tag{8.2}$$

where E_{cost} is energy expenditure, T_{fl} is the period of time spent in flight per foraging bout, T_h is the period of time spent handling each flower or floret of flower type per foraging bout, E_{fl} is the metabolic cost per second of travelling between nectar sources, and E_h is the metabolic cost per second of handling those sources. The energy expenditure per second can be calculated by dividing the right side of the equation by the mean length, in seconds, of the foraging bouts.

E_{fl} and E_h can be estimated in the laboratory using methods for measuring metabolic rates of insects when flying and stationary, respectively, at the range of temperatures experienced by the insects in the field (e.g., Acar et al., 2001). To measure E_{fl} insects can be allowed to fly, either tethered or free (e.g., Dudley & Ellington, 1990), within a respirometer, and their rate of oxygen consumption measured. The respirometer used by Gilbert (1983) to measure the oxygen consumption rate of loose-tethered *Eristalis tenax* was a paramagnetic oxygen analyser (other types of respirometer can be used, Acar et al., 2001). Alternatively, a 'flight mill' can be used and the amount of flight fuel consumed calculated. With flight mills, insects are tight-tethered to a balanced, lightweight arm, and are made to fly

continuously until they become exhausted (e.g., Akbulut & Linit, 1999). Gilbert (1983) used such a device to measure the rate of energy consumption in flying *E. tenax*, simply recording the amount of weight loss during flight, and, from the figure obtained, estimated the amount of carbohydrate (presumed to be the flight fuel) utilised and, thus, the amount of energy expended over the period of flight. Hocking (1953), by contrast, provided insects that had become exhausted on the flight mill with a known quantity of carbohydrate (glucose). The duration of the next 'flight' by the insect could then be measured and the rate of fuel consumption calculated. Tight-tethering in flight mills, because the insect does not have to support its own weight, may cause flight duration to be overestimated and thereby cause energy consumption to be underestimated. Even loose-tethering, as employed by Gilbert (1983) in respirometry, is likely to influence average flight performance, so affecting energy consumption. A better system may involve flight chambers that do not involve tethering at all (e.g., Asplen et al., 2009).

Measuring E_h could prove difficult. Heinrich (1975) suggested that the resting metabolic rates of nectarivorous insects approximate to the metabolic costs of feeding and also the costs of walking, so the practical problems associated with estimating the metabolic costs of feeding and walking may be conveniently avoided. However, this assumption needs to be tested for the insect species being studied. The metabolic rates of resting insects can easily be measured using respirometers (diurnal insects can be kept stationary in the dark).

If the nature of the metabolic fuel utilised by the insects under study is known, the energy required to sustain the observed metabolic rate at particular activities can be calculated. Such fuels may be carbohydrates, lipids and amino acids (e.g., proline) and the fuel used may vary according to the type of activity, and even within an activity type, such as during flight (Beenakers et al., 1984; but see Amat et al., 2012, for an example of invariant glycogen use by a parasitoid).

J: The net amount of energy that can be obtained from different flower types visited over a certain period of the day. Net energy, E_{net}, is the difference between gross energy intake (E_{in}) and energy expenditure (E_{cost}):

$$E_{net} = E_{in} - E_{cost} \tag{8.3}$$

May (1988) observed flower preferences in terms of E_{net}, which was used as the measure of 'profitability' of flower types. E_{net} is in fact the net energy value of a flower type and not its profitability, which is $E_{net}/(T_{fl} + T_p)$ (Stephens & Krebs, 1986). May (1988) asked whether the E_{net} of a flower type was largely a function of: (1) mean nectar volume; (2) mean nectar concentration, accessibility of the nectar (e.g., corolla length); (3) flower density; (4) flower dispersion; and (5) the density of florets per inflorescence, noting that (1) and (2) may vary with the time of day). May (1988) used multivariate statistical analyses to establish the main ways in which flower species differed with respect to these variables. As nectar volume explained nearly all of the variation in energy content among different nectar sources, it was concluded that nectar volume was the best single predictor of E_{net}, while nectar concentration and flower density were poor predictors. Multivariate analyses could also be carried out to determine what factors mainly determine the profitability sensu stricto of a flower type; handling time and flight time should be among the variables considered.

As noted earlier, relating flower selectivity to foraging profitability in pollen- and pollen-plus-nectar-feeding insects will prove more difficult than with nectarivores. One of the few possible advantages of studying pollen-feeders is that the amount of pollen consumed may be easier to quantify compared with the amount of nectar.

In considering the exploitation of non-prey and non-host food sources by natural enemies, it is also important to bear in mind that, as well as having to decide which types of food source (e.g., flower species) to visit and what materials to consume, foragers need to decide how to

allocate their time and energy between foraging for non-prey or non-host food *versus* foraging for prey or hosts on the other. Among many species of parasitoids and aphidophagous hoverflies, females forage for hosts or prey and for food in distinctly different parts of a habitat, and as a consequence must be incurring significant opportunity (from an oviposition standpoint) and energy costs compared with insects that can forage for non-host and non-prey foods in the same areas as their hosts or prey. The problem of choosing between ovipositing or feeding in patches of differing profitability (in terms of net energy and net fitness gain) is of importance from an 'applied' (conservation biological control, Sect. 8.6) as well as a 'pure' perspective. State-dependence of behaviour (Sect. 1.8) is a key consideration. The state-dependent optimal strategy, based on the amount of energy reserves, for a parasitoid faced with a choice of visiting host or food patches has been examined from a theoretical standpoint by Sirot and Bernstein (1996) using dynamic programming (Sect. 1.2.2). The Sirot and Bernstein (1996) model is based on pro-ovigenic parasitoids (i.e., energy reserves determine only life-span, not egg supply), a life-history extreme which appears to be exceedingly rare (Jervis et al., 2001). However, modifying a dynamic programming model so that it is applicable to synovigenic parasitoids (which mature eggs during their adult lives, dependant on energy reserves) is possible as well (Heimpel et al., 1998).

8.6 Pest Management Through the Provision of Plant-Derived Foods: A Directed Approach

There have been numerous cases where the incorporation of plant diversity within an agricultural system has led to a decrease in insect pest densities (reviewed by Risch et al., 1983; Russell, 1989; Andow, 1991; Bugg & Waddington, 1994; Coll, 1998; Gurr et al., 2000, 2016; Landis et al., 2000; Bommarco & Banks, 2003; Fiedler et al., 2008; Isaacs et al., 2009; Jonsson et al., 2010; ; Wyckhuys et al., 2013; Heimpel & Mills, 2017; Penalver-Cruz et al., 2019). One explanation for pest decrease is that increased plant diversity enhances the action of natural enemies of pests –this is the 'enemies hypothesis' of Root (1973) (reviewed by Russell, 1989, and Heimpel & Mills, 2017).

The basis of this hypothesis is that increased plant diversity provides natural enemies with resources that are in limited supply in monocultures; these resources include a favourable microclimate, alternative hosts or prey, and plant-derived foods (Jervis et al., 1992, 2004; Landis et al., 2000). During the last two decades, this topic has been reviewed in detail by Landis et al. (2000), Heimpel and Jervis (2005), Wäckers and van Rijn (2012), Gurr et al. (2017), and Perovic et al. (2018). Most of the studies have analysed the improvement of the effectiveness of parasitoids and predators through the provision of 'supplemental foods'. The latter take the form of nectar- and/or pollen-providing plants and artificial substitutes (e.g., sprayed solutions of sucrose, often incorporating nitrogenous materials, so-called 'artificial honeydews', reviewed by Wade et al., 2008; Tena et al., 2016).

Biological control practitioners intend that, by providing either natural or substitute foods, the local natural enemy population will be positively affected in one or, preferably, more ways: (1) the natural enemies will be attracted into the crop (i.e., food provision encourages immigration into pest-containing areas); (2) the natural enemies are retained within the crop (i.e., food provision discourages emigration from pest-containing areas); (3) changes occur in natural enemy life-history variables with the result that *per capita* searching efficiency and the predator's or parasitoid's numerical response are enhanced, such that, within the crop, the rate of parasitism or predation is increased, and that pest numbers are thus ultimately reduced to a desirable level.

In cases where plants are used to provide the supplemental or substitute foods (floral nectar, pollen, extrafloral nectar), they are:

(1) Deliberately sown. In most cases, the sown plants are not harvested (although they can be a species of crop plant), but in some

cases they can be harvested as part of an intercropping system (e.g., Gallego et al., 1983; Letourneau, 1987). Intercropping, as employed in this context, takes various forms (e.g., Schoonhoven et al., 1998; Lopes et al., 2016; Gontijo, 2019; Penalver-Cruz et al., 2019; Iuliano & Gratton, 2020): (1) mixed cropping–the growing of the two crops (pest-bearer and food-provider) with no distinct row arrangement; (2) row intercropping–the growing of the different crops in distinct, interdigitating rows; and (3) strip intercropping −the growing of the different crops in strips sufficiently wide to allow independent cultivation but narrow enough for the crops to interact (and therefore enable manipulation of natural enemies on the pest-bearing crop), or:

(2) Present naturally as 'weeds', which are tolerated or encouraged (e.g., Zandstra & Motooka, 1978; Altieri & Whitcomb, 1979; Andow, 1988; Bugg & Waddington, 1994; Bugg & Pickett, 1998; Nentwig, 1998; van Mele & van Lenteren, 2002; Gunton, 2011; Seguni et al., 2011; Araj & Wratten, 2015; Araj et al., 2019; Gontijo, 2019; Möller et al., 2021). In some cases, they are intended to serve as both a natural-enemy food source and as trap plants for diverting pests away from the crop (e.g., Leeper, 1974).

To increase the likelihood of success of a habitat manipulation programme, fundamental research should be conducted into natural-enemy resource requirements: it should provide information that will at best increase the chances of success of the programme and will, at the very least, provide valuable insights into natural-enemy biology (Jervis et al., 2004). Investigating the effects manipulation will have on the component members of a pest's natural enemy complex, as opposed to entire guilds or source subwebs, is advocated (Jervis et al., 2004), that is, in the terminology of Gurr et al. (2003), using a 'directed' as opposed to a 'shotgun' approach (see also Heimpel & Mills, 2017). This, it is argued, will not only increase the chances of obtaining a positive outcome of manipulation but will also provide a deeper understanding of the mechanisms responsible for failures as well as successes (see also the 'active adaptive management' approach advocated by Shea et al., 2002, for various problems in pest management). Through a directed approach, the gathering of even the most basic biological information (e.g., on whether a parasitoid needs to feed as an adult), can enable candidate species from among a pest's enemy complex to be compared and ranked with respect to pest control potential under a manipulation programme. The directed approach ought, ideally, to involve the research on several fronts in the following order:

1. *Establishing that the candidate natural enemies actually require, and will seek out, supplemental or substitute foods to a significant degree.* Species whose adults have vestigial mouthparts (Gilbert & Jervis, 1998; Jervis, 1998) will have no need for external nutrient inputs and are very unlikely to respond behaviourally or reproductively to food sources, so can be excluded from the list of candidate agents. Dissection of very recently emerged females, and measurement of the ovigeny index using the total number of oöcytes as the estimate of lifetime potential fecundity can provide a useful clue to the dependency of a parasitoid species on foods (see Sect. 2.3.4 for caveats). Females of pro-ovigenic species ought, theoretically, to have less need for food than synovigenic species. There are, however, exceptions; for example, while mymarids appear in general to be pro-ovigenic (Jervis et al., 2001), and are only occasionally observed feeding in the field (Jervis et al., 1993), a high proportion of field-caught individuals of one species contained sugars in the gut (Heimpel & Jervis, 2005). Similarly, the sugar content of two species of *Anagrus*, also pro-ovigenic mymarids, vary among seasons and time of the day (Segoli & Rosenheim, 2013). Although synovigenic insects will typically be more dependent on foods than will pro-ovigenic species, some can obtain nutrients

via feeding on host or prey haemolymph and/or tissues (i.e., 'host feeding', Jervis & Kidd, 1986; Heimpel & Collier, 1996), as has been demonstrated for the eulophid *Eupelmus vuilletti* (Giron et al., 2002, 2004). The latter physiological type of insects ought to be less sensitive to manipulation via the provision of sugar-rich non-host foods. Nonetheless, most parasitoids cannot achieve maximal fecundity by host feeding alone: females need to feed on non-host sugars in order to fully benefit from host meals (Heimpel et al., 1997a; Kapranas & Luck, 2008; Benelli et al., 2017) as the amounts of carbohydrates obtained by host feeding is very limited (Tena et al., 2013b).

2. *Demonstrating that food resources are truly limited in supply, both within and in the vicinity of the crop and thus that the natural enemy is sugar limited.* Demonstrating that natural enemies are sugar limited can be accomplished by the use of biochemical techniques (Sect. 8.2.3). While it is a valid generalisation that nectar is in short supply in monocultures, natural enemies can still feed on honeydew. For example, olive orchards in southern Spain are devoid of flowering plants (Jervis et al., 1992; Jervis & Kidd, 1993), but the hemipteran *Saissetia oleae*, the black scale, which is present in the crop throughout the year, excretes honeydew of high nutritional value for natural enemies and other insects (Wang et al., 2011; Villa et al., 2016). Other crops, such as alfalfa and citrus, also support populations of hemipterans (which may or may not be the target pest) that produce honeydew on which natural enemies feed (e.g., Evans & England, 1996; Tena et al., 2013b, 2016; Rand & Lundgren, 2018). However, the value of such honeydew may be limited if it is nutritionally poor or if it occurs in insufficient quantities (if the population density of the producer is low), or if natural enemies compete with ants for access to it (Tena et al., 2013b, 2016; Calabuig et al., 2015). The crop may also produce extrafloral nectar that parasitoids can feed on (Heimpel & Jervis, 2005; Röse et al., 2006). In fact, parasitoids are, after ants, the second most commonly reported insects feeding on extrafloral nectar (Heil, 2015).
3. *Determining the plant-based diet breadth and feeding preferences of the natural enemy.* Once it has been demonstrated that natural enemies need food sources, and that they are in short supply, it is necessary to select an appropriate food source for the selected natural enemy. As already mentioned (Sect. 8.3), mouthpart morphology can be very useful in this respect. Some species of flower-visiting natural enemies exploit a very wide range of flower species (e.g., Tooker & Hanks, 2000); a high degree of specificity is shown in others, particularly some bee-flies (Sect. 8.3).
   To assess preferences, field counts can be made of foragers (e.g., on different resources, Cowgill et al., 1993; Colley & Luna, 2000); ideally, these should be related to the abundance of the resource (Sect. 8.4). Research into the preferences that natural enemies have for certain food types, including nectar constituents, can create opportunities for the selection of food supplements (whether natural or artificial) specifically targeted at the natural enemy (e.g., Potter & Bertin, 1988; Lanza, 1991; Gurr et al., 1999). Such work, particularly when combined with investigations that examine the effect particular constituents, or combinations thereof, have on key life-history variables of both natural enemies and pests (e.g., longevity; Wäckers, 2001), can identify those food sources that should be excluded due to their positive effect on the pests (see below).
4. *Evaluating the attraction of supplementary foods to natural enemies.* Olfactometry and other behavioural assay techniques (Sect. 1.5.2) can be used to establish and compare the attractiveness of flowers. They can also help in maximising the attractiveness of artificial honeydew mixtures (for protocols, see van Emden & Hagen, 1976; Dean & Satasook, 1983; McEwen et al., 1993). It is important that the state-dependence of behaviour be considered in the experimental

design (e.g., Lewis & Takasu, 1990; Wäckers, 1994; Hickman et al., 2001, sect. 1.3). For example, recently fed natural enemies are not likely to be strongly attracted to food sources, nor are females that have a high egg load (Jervis et al., 1996).

Knowledge of the attractiveness of a food, of the nature of the stimuli involved, and of the dispersal capabilities of natural enemies, can shed useful light on: (1) whether immigration can be significantly enhanced by food provision; a principal aim of manipulation is that attraction, either acting alone or in combination with arrestment will result in within-crop aggregation at the population level (Sect. 1.15); (2) the potentially most effective spatial arrangements (e.g., different intercropping regimes, see above) of the supplemental/substitute food resources relative to crop plantings. Synthetic herbivore-induced plant volatiles (HIPVs) combined with floral resources have been tested to increase the recruitment of natural enemies and feed them. This combined application, named 'attract and reward', has shown variable results because, although both of these approaches offer potential to enhance biological control separately, their synergistic effect its unclear (Simpson et al., 2011; Kaplan, 2012; Gordon et al., 2013).

5. *Evaluating the effect of supplementary foods on the life-history variables in such a way as to increase the host or prey death rate and, if a consideration, the natural enemy's rate of increase.* This includes measuring the effects of supplemental feeding on reproduction, growth, development, survival and fecundity, and also sex ratio. Protocols, some of which can also involve measurement of functional responses (see 10, below) can be found in Chap. 2. Ideally, both lifetime realised fecundity and longevity should be measured (see 7, below), involving at least the range of host or prey densities that characterise field conditions, and with non-host or non-prey foods present and absent. If the use of a range of densities is not possible, predators and female parasitoids should be given a superabundance of hosts (this also applies to measurements of maximum attack rates). Researchers concerned only with longevity should consider the likelihood that for some, if not most, parasitoids (even including pro-ovigenic species) oviposition incurs a life-span cost (Jervis et al., 2001), and that host-deprived females of some synovigenic species may obtain nutrients for somatic maintenance by resorbing eggs. Longevity estimates made for host-deprived females may therefore be strongly biased and poorly indicative of the field situation. Lastly, life-span needs also to be considered in relation to mortality factors unrelated to nutrition. For example, if predation of adult parasitoids is so strong that life-span is reduced to starvation levels in the field (Heimpel et al., 1997b; Rosenheim, 1998), food provision will have little impact on life-span in the field (Heimpel & Jervis, 2005; Heimpel & Casas, 2008).

   Increased longevity of natural enemies can be important during periods when host or prey resources are absent, for example, during the period following harvesting and prior to reseeding or the planting of the crop. For some predator and parasitoid species, supplemental foods may serve only as 'stop-gap' resources (Gurr et al., 2003), enabling survival (and a high search rate) over a short time period, whereas for others they may permit a substantial amount of development, reproduction and survival to occur in the absence or scarcity of prey (e.g., Smith, 1961, 1965; Kiman & Yeargan, 1985; Cottrell & Yeargan, 1998; Crum et al., 1998; van Rijn & Tanigoshi, 1999; Wäckers, 2005; Beltrá et al., 2014).

6. *Demonstrating that the natural enemies feed on the selected materials under field conditions.* Whilst a supplemental food source may have been shown to be attractive in the laboratory, additional experiments will need to be conducted to establish that food location is not seriously constrained, under field conditions, as a result of interference from factors associated with other vegetation,

including the pest-bearing crop. Presence of odours may disrupt olfactory responses (e.g., Shahjahan & Streams, 1973). Moreover, the natural enemy may not be amenable to manipulation if individuals are incapable of, or are otherwise poor at, travelling to the food source and in moving from the food source to the pests within the crop. This may apply particularly to small-bodied, apterous or brachypterous species.

The ability of natural enemies to feed on the provided foods can be determined by direct observation (Sect. 8.2.2) and/or by the use of dissection, biochemical techniques and food labelling (Sect. 8.2.3). In the case of biochemical techniques, it can be difficult to determine whether natural enemies are feeding on the provided alternative food source or other food sources available in the field (Lee et al., 2006). Laboratory behavioural tests alone are insufficient also because of the presence of competitors in the field (Lee & Heimpel, 2003; Lee & Calabuig et al., 2015), as well as extremes of temperature and humidity that may affect the suitability of the food source (Winkler et al., 2009). For example, honeydew and artificial sugars can crystalise under warm and dry conditions (Wäckers, 2000; Wade et al., 2008). Researchers should also take into consideration that the requirements may vary among seasons, as has been demonstrated for mymarid and aphelinid parasitoids (Segoli & Rosenheim, 2013; Tena et al., 2013b). Therefore, the nutritional status should be measured at different periods, especially during those when nectar and honeydew may be scarce or not accessible (Tena et al., 2016).

7. *Demonstrating that parasitoid fitness increases in the field*. Even if a supplemental food source is shown to be effective in promoting development, reproduction and survival in the laboratory, and it is shown that it is used in the field, it does not necessarily follow that parasitoid fitness will be increased in the field. Confounding factors include rainfall, extremes of temperature and humidity, and bacterial or fungal contamination (e.g., Sikorowski et al., 1992). In pro-ovigenic parasitoids, an increase in realised fecundity can be measured by counting the number of eggs in females collected in the field soon after they died naturally and comparing with the number of eggs of newly emerged females (Segoli & Rosenheim, 2013). In synovigenic parasitoids, two numbers are needed: the realised fecundity per day, which can be measured by counting the number of eggs inside parasitoids that were caught at the beginning or at the end of the day, and the number of eggs that they mature per day, which can be measured by maintaining female parasitoids under controlled conditions (Casas et al., 2000; Lee & Heimpel, 2008; Dieckhoff et al., 2014; Tena et al., 2015). Measuring longevity in the field is likely to be more challenging. For parasitoids, wing wear indices using counts of broken setae on the fringe of the forewing as an estimation of longevity can used for some species (Heimpel et al., 1996; Lee & Heimpel, 2008; Miksanek & Heimpel, 2020).
8. *Demonstrating that*, *within the current pest generation*, *the supplemental food does in fact increase the densities of natural enemy within the crop*, *whether by attraction*, *by arrestment*, *or by both processes* (see Chap. 6 for protocols on estimating parasitoid and predator densities). Note that a high degree of attractiveness of a food source does not necessarily translate into increased natural enemy densities (e.g., Bugg et al., 1987) and that an increase of natural enemy densities can also be the result of an increase of their longevity (Tylianakis et al., 2004; Tena et al., 2015).

Arrestment will occur by virtue of the natural enemies visiting, probing, and feeding at food sources. However, there may be additional arrestment effects, acting through searching movements. Artificial honeydews are likely to act as arrestants in the same way as natural ones, resulting in alterations in search paths (and increasing the rate of egg deposition) (Carter & Dixon, 1984; Ayal,

1987; Budenberg, 1990; van den Meiracker et al., 1990; McEwen et al., 1996). Thus, when using artificial honeydews, consideration needs to be given to the possibility that a powerful formulation, in arrestment terms, or a weak spatial association between spray deposits and host patches, may confound the manipulation by constraining natural enemy searching efficiency. Using paint marking, coupled with intra-strip counts of adults and measurements of dispersal rates from strips, MacLeod (1999) was able to assess the effectiveness of field-margin vegetational strips of different floral richness in retaining adults of a beneficial hoverfly.

9. *Establishing the potential for supplemental food to increase the natural enemy's numerical response*. Recruitment into the subsequent generation of the natural-enemy population may be a key consideration in those manipulation programmes in which the pest is present in the crop for longer than the duration of one parasitoid or predator generation.

   The numerical response depends largely on three components: development rate, larval survival, and the realised fecundity of females, all of which can be positively influenced by food provision (see 7, above). In anautogenous natural enemies (adult females must feed on a host or prey before maturing and laying eggs) the numerical response can also be positively affected due to the lowering of the threshold prey density at which eggs can be laid. Glasshouse and cage experiments can provide valuable information on the effects of supplemental food on the numerical response, as the confounding effects of immigration and emigration can be ruled out. Using a glasshouse experimental set-up, van Rijn (2002) showed that pollen provision can greatly improve the control of thrips by predatory mites (in this case, despite the fact that the pollen was fed upon by the prey as well as the predator). Improved control results because the predators' numerical response to pollen and thrips density outweighs the two other effects of pollen-feeding: the negative effect of a reduction in the predator's functional response, and 'positive' effect of an accelerated pest population growth rate.

   For supplementation to have a useful effect in the vicinity of the floral strip or other manipulation, natural-enemy numerical responses or fitness increases must be realised locally. However, some parasitoids may engage in medium- or long-range dispersal upon obtaining a sugar meal. Under the sugar-fuelled dispersal hypothesis, parasitoids use sugar meals to enable strategies in which offspring are dispersed among widely separated host patches. Potential selective forces that would favour sugar-fuelled dispersal include the avoidance of self-superparasitism, density-dependent hyperparasitism or inbreeding, among other factors (Heimpel, 2019). While such strategies may reduce the local effects of sugar supplementation, they presumably still increase the fitness of parasitoids and therefore parasitism rates at a broader spatial scale.

10. *Demonstrating that food provision has the potential to affect the prey or host death rate.* A parasitoid's functional response to host density will vary with female fecundity and egg maturation rate (Chap. 1). Non-host food provision may alter the shape of the functional response curve in parasitoids, raising parasitism in cases where age-specific potential fecundity (egg load) is limited by external nutrient availability. The parasitism inflicted by each individual female parasitoid on a host population over her lifetime will be influenced by variation in age-specific fecundity and by life-span; both of these life-history variables will alter with the availability and the quality of energy-rich foods. Lifetime functional response experiments are therefore recommended (for protocols, see Bellows, 1985; Sahragard et al., 1991, and Chap. 1). In predators, supplemental food provision might result in a decreased *per capita* predation rate because the supplemental food enables insects to meet their nutrient requirements for growth and

development, egg production and survival. Such reduction of predation has been observed in different families of predators under laboratory conditions (Lundgren, 2009, provides examples on lacewings, phytoseiid mites, anthocorids and coccinellids). This potential negative effect may be offset by higher levels of fitness among predators that eat a diversified diet (Rijn, 2002).

11. *Demonstrating that food provision brings about a significant reduction in pest densities, e.g., to a level below an economic spray threshold* (Tena et al., 2015).
12. *Finally, when undertaking the ranking of potential food sources, consideration needs to be given to ease of cultivation and the potential for undesirable side-effects*. The latter include an unacceptable level of invasiveness (i.e., weed status needs to be considered), positive effects on the pest (adults of lepidopteran pests, for example, may feed at, and benefit reproductively from the flowers; Baggen & Gurr, 1998; Baggen et al., 1999; Romeis & Wäckers, 2000; Wäckers et al., 2007), and positive effects on hyperparasitoids or other higher-level predators associated with the natural enemy that is to be manipulated (Stephens et al., 1998; Goelen et al., 2018; Tougeron & Tena, 2019). An additional consideration in ranking food-providing plants is the potential for interactions with other visitors (such as bees and ants) arising from interference or resource exploitation (Lee & Heimpel, 2003; Calabuig et al., 2015).

8.7 Natural Enemies as Pollinators and the Use of Flower Visitation Webs

When visiting flowers to feed, aphidophagous hoverflies become contaminated with pollen over their body surfaces (Stelleman & Meeuse, 1976; Doyle et al., 2020). Although the amount of pollen carried by a fly in this way is probably small compared with that carried by non-predatory and hairier relatives such as *Eristalis tenax,* 'the common drone fly' (a species of hoverfly) (Holloway, 1976), it has been demonstrated that hoverflies can increase pollination and fruit production in sweet pepper (Pekas et al., 2020; Moerkens et al., 2021). Due to the flower constancy of individuals, even generalist hoverfly species may be effective pollinators (McGuire & Armbruster, 1991; Inouye et al., 2015; Rader et al., 2016). The same can be said for parasitoid flies, particularly tachinids (Al-Dobai et al., 2012; Wiesenborn, 2015; Glinos et al., 2019; Skaldina, 2020; Martel et al., 2021).

Parasitoid wasps, particularly those visiting flowers with narrow tubular corollas, are likely to come into accidental contact with the anthers (if not actually feeding on the pollen, Jervis et al., 1993; Gilbert & Jervis, 1998; Jervis, 1998) and so pick up pollen grains. Whether parasitoid wasps are significant pollinators will depend on (1) them visiting more than one individual plant during a foraging bout; (2) whether they show some degree of flower constancy, and (3) whether the pollen-bearing part of the body surface comes into contact with stigmas. These are aspects of parasitoid wasp behaviour about which we know very little. Most knowledge pertains to wasps having an association with orchids, involving pseudo-copulation by the males: certain Tiphiidae, Scoliidae and pimpline Ichneumonidae visit the orchid inflorescence solely for the purpose of mating, the orchids providing no food (e.g., van der Pijl & Dodson, 1966; Stoutamire, 1974; Kullenberg & Bergstrom, 1975). It is interesting to note that the book *British Flora*, published by Clapham et al. (1989), does not mention parasitoids specifically as flower-visitors. The role of parasitoids in pollination is an area that clearly has been neglected, perhaps due to:

1. The very small size and relative inconspicuousness of many species;
2. The tendency of some of the larger parasitoids not to linger at inflorescences in the manner of some bees, butterflies, beetles and hoverflies (Hassan, 1967; Jervis et al., 1993);
3. The often immense difficulties associated with their identification.

It is possible to demonstrate that an insect is capable of cross-pollinating, by depositing dyed, or otherwise labelled, pollen grains on inflorescences in anthesis, and determining whether these identifiable grains appear on the stigmas of other inflorescences. Stelleman and Meeuse (1976) used this approach and established that the aphidophagous hoverflies *Melanostoma* and *Platycheirus* do transfer pollen between spikes of *Plantago lanceolata*. Pollination efficiency of insects can be measured according to the protocols given in Dafni (1992) and Kearns and Inouye (1995).

Memmott (1999), a pioneer in the construction of quantitative parasitoid–host food webs (Sect. 6.3.12), applied similar methodology to flower-visitors to construct a quantitative flower visitation web for 26 species of flowering plant and 79 species of insects, among which were several aphidophagous hoverflies. By incorporating data on pollination efficiency, visitation webs can be developed into pollinator webs (e.g., Traveset et al., 2015). The latter could be used to identify which insect natural enemies are useful as pollinators as well as biological control agents. The information gained may be useful both in agriculture (e.g., maximising seed and fruit yields) and in plant conservation (e.g., habitat restoration) (Memmott, 1999).

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Statistical Approaches

9

Ian C. W. Hardy and Daniel R. Smith

9.1 Introduction

Entomologists often want to test hypotheses (Popper, 1968) or quantify the magnitude of effects, and this can be accomplished using statistical models. Statistics is the study of variation. It is not surprising that entomologists employ statistical methods to analyse their data, since in biological systems variation is the rule. This chapter uses words, diagrams and just a few very basic mathematical formulae to explain how statistical models work. We first provide an overview of foundation material that underpins the statistical framework that most scientists use (known as the frequentist approach; the alternative being the Bayesian approach which we do not discuss here; see Wilkinson et al., 2016, and Aguirre et al., 2021, for natural-enemy examples and Fornacon-Wood et al. (2022) for an entry into the fundamental differences between these paradigms). We focus on parametric statistical models. The most important message is that these models form a family of closely interrelated models which all work conceptually in the same way. The family is called generalised linear models (GLMs, sometimes also called GLZs, to distinguish them from the sub-family of general linear models) and can be used to test hypotheses, quantify the magnitude, direction and precision of effects, and make predictions.

We note that it would take a large book, rather than a single chapter, to cover in depth and detail the range of statistical approaches that are available for, and relevant to, research on insects as natural enemies, and we have not attempted that here. This chapter is pitched at an introductory level, assuming the reader to have little prior statistical knowledge, but also with the aim of equipping the reader to be sufficiently conversant with relatively advanced analytical approaches to be able to then go on to acquire further statistical skills. We also use schematic figures as illustrations that are intended to assist understanding rather than to represent accurately the formats of figures that would appear in research reports. Further, we have not written this chapter only for those studying natural-enemy biology: our text is largely 'taxon free' and the key illustrations use imaginary, non-entomological, examples. Nonetheless, the background to this chapter is firmly rooted in analyses of data on parasitoids and other natural enemies and the approaches and examples we discuss should be readily applicable

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to these study systems. We do give references to examples from natural-enemy studies but without attempting to be exhaustive.

9.2 Foundations of Statistics

9.2.1 Descriptive and Inferential Statistics

The foundations of modern statistical theory were built in the twentieth century, largely from the works of the English statistician Sir Ronald A. Fisher (Hald, 1998). Though diverse in its scope, statistics can perhaps be summarised by just three words: 'descriptions', 'differences' and 'associations'. Descriptive statistics, e.g., the mean (a measure of central tendency, or location) and standard deviation (a measure of spread), are estimates of population parameters (fixed and usually unknown quantities) important for summarising raw data. Raw data are a collection of numbers (or possibly non-numerical responses) from an experiment or observational study that have not been transformed in any way.

Describing properties of data sets is very useful and it is these descriptive statistics that are commonly displayed in tables and figures in scientific publications. Often, however, we want to increase our understanding of patterns in our sample data by asking questions such as: 'is *A* different from *B*?'; or 'are changes in *C* associated with changes in *D*?' Assessing whether there are differences or associations is performed using inferential methods, which form the focus of this chapter. By using probability theory, we can make inferences about the population using samples at our disposal.

9.2.2 Hypothesis Testing

Statistical tests, either directly or indirectly, involve the formulation of hypotheses, which are tested and either retained or rejected. Under the null hypothesis (H_0), we initially regard any observed difference or association as being due to chance variation. For instance, in an experiment exploring the potential effect of different diet treatments on the subsequent adult size of developing insects, H_0 might be stated as: There is no underlying effect of treatment on adult size. In contrast, the alternative hypothesis (H_1) implies any difference or association is due to a real effect, i.e., there is an effect of treatment on the size of insects. Hypothesis testing is fundamentally about finding the most parsimonious (simplest adequate) description of our observations. The decision is usually made by comparing the value of a test statistic with some pre-determined critical value for a given significance level (Sect. 9.2.3) and degrees of freedom (df). The df of a statistical test is usually the sample size (n) minus the number of parameters estimated from the data. If the test statistic is larger than the critical value, then we can conclude the difference or association is 'statistically significant'. Conversely, if the test statistic is smaller than, or equal to the critical value, we conclude there is no statistical significance (NS). Not too long ago, critical values had to be looked up manually in published tables (a whole book of such tables is provided by Rohlf & Sokal, 1995), and *P*-values would be reported as less than a given value, e.g., <0.05 or <0.01. However, there are now numerous statistical software packages (e.g., GenStat, Prism, SAS, SPSS, R) that perform hypothesis tests for us (almost instantaneously with small to medium sized datasets) and provide the corresponding *P*-value. However, one still needs to have an understanding of what test to use, how the test works, and how to interpret the results.

Hypothesis tests are widely employed among biologists, though as we will briefly discuss, they are not without their critics (Sect. 9.8.1). Under the frequentist framework, a hypothesis test computes a test statistic; that is a standardised number summarising an effect (a difference or an association) in the sample data. For instance, the two-sample *t*-test computes the test statistic '*t*', which follows Student's *t* distribution under the null hypothesis (H_0) that the underlying population means are equal. The computed test statistic has a corresponding *P*-value, which represents the probability of observing an effect in the sample data, under the assumption that no such effect exists in the underlying population (i.e.,

that H_0 is true). Importantly, it provides evidence against H_0, and this becomes more so as the *P*-value decreases. If the computed *P*-value from a hypothesis test is less than an arbitrary threshold (typically 0.05) set a priori (i.e., before the test was carried out), then H_0 is rejected in favour of the alternative hypothesis (H_1) and it is reported that a 'significant' effect was detected.

Among life science journals at least, it is not uncommon to see the Results section of published articles littered with *P*-values. However, there are a number of common misconceptions concerning *P*-values. The first is that the *P*-value is the probability that H_0 is true. The second is that the *P*-value is the probability of rejecting a true H_0 (Type I error). The third is that the lower the *P*-value, the stronger the effect size. All of these statements are incorrect, since under the hypothesis testing framework, we are already assuming that H_0 of no effect is true, so it follows that computing a *P*-value in favour of it is illogical. Furthermore, there are a number of criticisms of hypothesis testing. The first is that the H_0 is hardly ever absolutely true (i.e., there is likely some true effect even if it is extremely small). Second, as sample size increases, so does the statistical power of a test, and accordingly, if a large enough sample is obtained, even tiny effects become 'significant': such a 'significant' effect may or may not represent biologically meaningful effects. Third, the threshold for significance is completely arbitrary; for instance, a 'significant' *P*-value of 0.049 is really not that much different to a 'non-significant' *P*-value of 0.051. The suggestion that, historically at least, significant *P*-values are more likely to secure publication (i.e., the infamous file-drawer problem, Yang et al., 2023) might only serve to encourage an improper habit to perform several hypothesis tests in the hope of obtaining an elusive 'significant' *P*-value. So-called 'fishing for significance' has long been recognised as a problem by statisticians. Despite these criticisms, *P*-values are probably here to stay (but see Halsey, 2019), and if used correctly, can be a valuable accessory in the day-to-day toolbox. However, in most forms of scientific inquiry, *P*-values alone reveal only part of the picture, and, for this reason, many scientific journals now recognise the importance of reporting a measure of effect size in addition to *P*-values.

An effect size is a point estimate of the magnitude and direction of an effect (e.g., difference between means, slope of a regression line) in the underlying population. This can be presented as a raw (e.g., a regression coefficient) or standardised (e.g., coefficient of determination, r^2) metric; the latter being normalised by the variability in the sample data. Invariably, the effect size is supplemented by an estimate of precision; that is, e.g., a confidence interval (CI). For instance, an effect size of 2.0 (95% CI: 1.5, 2.5) indicates that in the long run (i.e., if the experiment were repeated many times, each time re-calculating the confidence intervals), 95% of the time the population effect size would lie somewhere between 1.5 and 2.5, with 2.0 being our best estimate. The effect size has an assumed value under the H_0 and is therefore inextricably linked to the *P*-value. For example, Cohen's *d*, an effect size which quantifies the standardised difference between two means, has a value of 0 under H_0 since we assume the population means are equal. Therefore, if we pre-specify the significance threshold at 5%, the difference between the two means will be 'significant', if and only if, the 95% confidence interval does not include zero.

9.2.3 Probability and Statistical Significance

Probability is quantified by a number between 0 and 1; with 0 implying no chance of an event and 1 that the event is certain. Probability is thus a proportion and can therefore also be expressed as a percentage (%) out of 100; e.g., a proportion of 0.5 indicates a 50% probability of the event occurring. A significance level refers to the acceptable level of probability that any effect we observe is due to chance alone (i.e., that samples drawn from a randomly varying set of responses just happen to give the appearance of an underlying effect). The choice of a particular significance level is an ultimately arbitrary convention, though it is often taken to be 0.05 (i.e., 5%) as

suggested by Fisher (Sect. 9.2.1). Thus, we reject H_0 if the probability that an observed pattern in the data could have arisen by chance alone is estimated to be <5%. For example, when evaluating an observed difference in the size of adult insects between treated and untreated samples, if $P < 0.05$ then the difference would be 'statistically significant' and we can make concluding statements such as 'there was a significant difference in the size of insects between treated and untreated samples ($P < 0.05$)'. Conversely, if $P > 0.05$ then the difference would be non-significant (NS). The lower the probability, the more evidence we have against H_0 that the observed effect is just due to chance.

9.2.4 Statistical Errors and Power

When H_0 is rejected, H_1 is supported. When we fail to reject H_0, then H_0 is supported. In both cases, however, we have not 'proved' anything since the sample data might be compatible with several hypotheses. When we decide to use the 5% significance level, we are also accepting that sometimes we will draw incorrect conclusions. It may be that we conclude a significant difference in insect size from our sample data, but in reality, no such effect exists in the underlying population. Rejecting a true H_0 is termed a Type I error (Table 9.1). Conversely, if we had not detected a significant difference from our sample data, this would not guarantee that there is no underlying effect in the population. Failing to reject an incorrect H_0 is a Type II error (Table 9.1). The probability of committing a Type I error is termed α (note this is also our significance threshold) and the probability of committing a Type II error is termed β. The statistical power of a test (e.g., Smith et al., 2011; Yang et al., 2023) is the probability of rejecting H_0, given that there really is a genuine effect (i.e., H_0 is false). Thus:

$$\text{Statistical power} = 1 - \beta \qquad (9.1)$$

Table 9.1 Underlying truth and analytical conclusions from null-hypothesis testing (after Quinn & Keough, 2002). There are two types of errors associated with null-hypothesis testing. Imagine an experimental situation where we are trying to decide whether there is an effect or not. A Type I error (α) occurs where really there is no effect, but one was erroneously detected in the sample data. A Type II error (β) occurs when there really is an effect but the test employed was not powerful enough to detect it. Values of $\alpha = 0.05$ and $\beta = 0.2$ are often considered acceptable, though there are no hard and fast rules

Truth (population)	Conclusion (based on sample data)	
	Reject H_0	Retain H_0
Effect	Correct decision	Type II error
No effect	Type I error	Correct decision

9.2.5 Parametric and Non-parametric Statistics

Hypothesis testing methods usually fall into one of two major categories: parametric and non-parametric (although there are also semi-parametric methods). Non-parametric tests make few or no assumptions about the underlying distribution of data values (Neave & Worthington, 1988; Siegel & Castellan, 1988; Dytham, 2011). This makes them applicable to a wide variety of situations. However, they are typically less powerful than parametric tests. They are also typically less flexible in terms of how they can be combined to test several hypotheses within the same analysis: non-parametric tests tend to be used to address one H_0 at a time and not to assess interaction effects (Sect. 9.5).

In contrast, parametric tests assume the data conform to some underlying distribution. If we imagine a population of animals in which older individuals tend to be larger (as in larval stage lepidopterans, or elephants, Briffa et al., 2013), then the underlying truth is that there is a positive relationship between age and size. However, not

every individual in the population will conform exactly to this relationship (some will be small for their age and some will be large for their age due to other sources of variation) so there will be a distribution of sizes around any particular age class. The differences between the sizes of individuals and the underlying relationship for the population are known as 'errors', and parametric statistical tests rest on assumptions about the way these errors are distributed. Note that the meaning of 'errors' here is different from that used to indicate interpretational or methodological mistakes (Table 9.1) and often the closely related term 'residuals' is used instead: residuals are differences between an observed value and a fitted statistical relationship in the sample data, whereas errors are differences between an observed value and the true underlying relationship in the population.

Statistical testing is almost never carried out on entire populations (even if we could assess all individuals in a population at a given time this would not necessarily indicate underlying effects in the populations because populations are continually changing in composition due to births and deaths), so tests rely on data which has been sampled from the population. Relationships between characteristics such as age and size may also be present in samples and the difference between the value of a sampled data point and the relationship within the sampled set of data is, as mentioned above, known as a residual (Sect. 9.3.1). Parametric statistics thus make assumptions about distributions of errors but operate by calculating residuals (see below).

Different parametric methods assume different distributions of errors, but the methods are all related and many of the assumed distributions belong to the 'exponential family', which includes the normal (also called Gaussian or 'bell-shaped'), Poisson, binomial, negative binomial, Weibull and gamma distributions. This chapter is concerned with analysis of data sets in which the initial assumption is that data values underlyingly conform to one of these distributions and our focus is on the normal distribution and two non-normal distributions: the Poisson and the binomial (Fig. 9.1). The Poisson distribution is used in analyses of data on integers (counts) that range from zero upwards to, potentially, infinity (Sect. 9.7.1) and the binomial distribution is used in analyses of binary events that give rise to proportions (Sect. 9.7.2). We very briefly discuss the use of other distributions in Sect. 9.8. Models that assume the distribution of errors to be normal are termed 'general linear models' and those that assume that the errors conform to one of the wider class of distributions within the exponential family are termed 'generalised linear models' (of which general linear models are a special case).

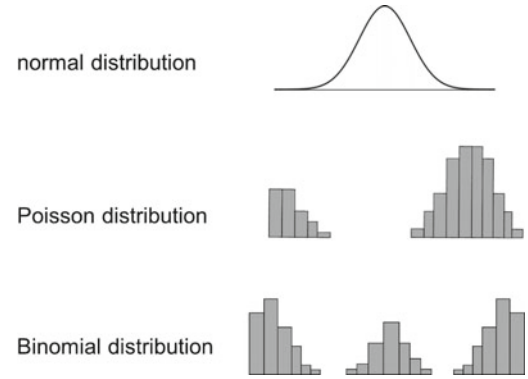


Fig. 9.1 Summary icons we use for three important distributions. The normal or Gaussian distribution is bell-shaped and is symmetrical about its mean (which is also equal to its median and mode). The Poisson distribution has a bell-like shape when the mean is large but lacks the left-hand tail when the mean is small. The binomial distribution also changes shape according to the mean. The left-hand tail is lacking when the mean is low whereas the right-hand tail is missing when the mean is high. The icons for the Poisson and binomial distributions take the form of histograms (one bar for each integer) because these distributions deal with values based on whole number counts, while the normal distribution concerns continuous variables (including non-integer and negative numbers)

9.3 Standard Regression

We have now covered general issues that underly various statistical approaches and here we turn to discussion and explanation of particular parametric techniques. We begin, in this section, with regression, next we cover analysis of variance (Sect. 9.4), and then we show how they can be

combined (Sect. 9.5) and how the combination of statistical modelling techniques with apparently very different names is straightforward, flexible and extremely useful (Sect. 9.5). In explaining regression, we cover aspects, such as parsimony and coefficients of determination, that are also employed in the models we cover subsequently. This should be unsurprising since we have already noted that these models are members of a broader family (generalised linear models) and all work conceptually in the same way. What we are working towards is an understanding of this family of models that is summarised in the final figure of the chapter: skip to the end and look at it if you wish, but it will ultimately make more sense if the explanation is built step by step. Before proceeding with the topic of regression, we note that we could have equally opted to first explain analysis of variance, and then discussed regression and then how these techniques can be combined. We chose to cover regression first simply because it seems slightly easier to visualise using schematic figures.

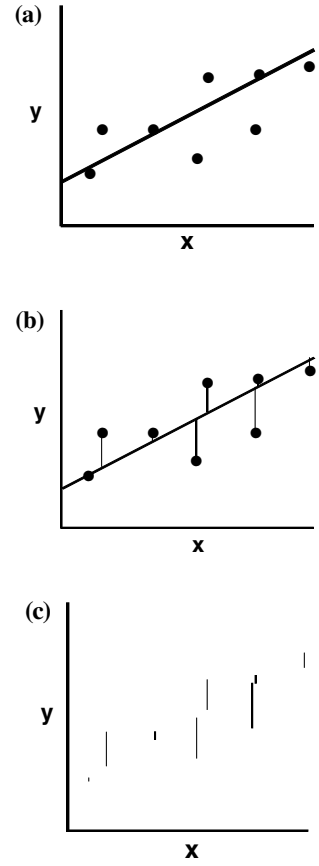
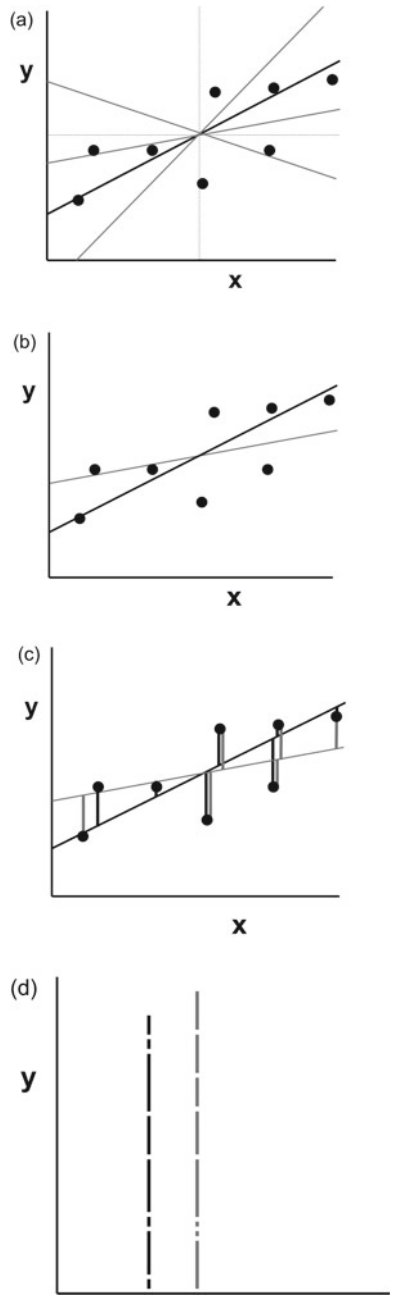


Fig. 9.2 Regression lines and residuals. Panel **a** line fitted through observed data points. Panel **b** show the same data but with residuals emphasised. Panel **c** residuals only shown: it is not standard practice to show residuals but we use them here and in the following figures to illustrate the statistical methods. Note that an equivalent example could be given for a negative (downward-sloping) relationship rather than a positive (upward-sloping) relationship

9.3.1 Sum of Squares (SS)

Standard regression (also known as ordinary least-squares regression) can be formulated as a general linear model to explore the association (relationship) between two continuous variables under several assumptions: in other words, we wish to explore the effect of one quantitative trait on another (Box 9.1). We will take a graphical approach (following Crawley, 1993). The response variable (sometimes called the dependant variable) is plotted on the *y*-axis (vertical) and the explanatory variable (sometimes called the independent variable, or predictor variable) on the *x*-axis (horizontal) of a standard two-dimensional plot. The essence of standard regression is to estimate the parameters which define the true 'best fit' for the underlying population (Fig. 9.2a). Unless the sample data points happen to lie perfectly on a straight line, which is rather unlikely in the biological sciences, some data points will be further from the fitted line than others. The vertical difference between an observed data point and the fitted line is the residual (Fig. 9.2b, c). The best-fit line should pass through the point where the sample means of the response and explanatory variables are located and can be conceptualised as rotating the line about this point until the residuals are minimised (Fig. 9.3).



◀ **Fig. 9.3** A visual illustration of finding the best-fit line in regression. **a** The best-fit line should pass through the point where the mean of *y* and mean of *x* coincide and can be 'found' by rotating the line about this point until the residuals are minimised. **b** Imagine we have narrowed our choice down to just two candidates, the solid black line and the grey dashed line. **c** Each of these lines has a set of residuals associated with it, a black set and a grey set. If we remove the data and the lines from the graph in (**c**) and just concentrate on the residuals, all we need to do is add up the total lengths of each set to see which is shorter. **d** If we imagine picking the residuals up and putting them into two piles (and ignore that in this visualisation the *x*-axis no longer has meaning), it becomes clear that the sum of the black residuals is smaller so the black line is the better fit to the data. Finding the best-fit line actually involves this process for every possible candidate line, and there are a myriad of these. Fortunately, nowadays we can let computers perform these calculations for us. Note that when done mathematically rather than visually, the residuals would actually be squared before being added

Box 9.1 Assumptions of General Linear Models

1. Values of explanatory variables are known without error.
2. The response variable is potentially affected by the explanatory variable, but not vice versa.
3. Data points are mutually independent.
4. The response variable is normally distributed and has constant variance.

General linear models include regression, multiple regression, *t*-tests, ANOVA, ANCOVA and factorial ANOVA and are a sub-family within the wider family of parametric statistical tests known as generalised linear models (GLMs). When errors are normally distributed, residuals

from a fitted GLM should be approximately normal.

The assumptions of GLMs are the same as for general linear models, except that the assumption of normally distributed errors is broadened, such that errors may be assumed to conform to some other distribution within the exponential family. Thus, for instance, log-linear models assume Poisson-distributed errors, whereas logistic models assume binomially distributed errors. Note that Poisson and binomial distributions of errors do not necessarily lead to Poisson and binomial distributions of residuals.

Assumptions 1 and 3 and are also assumptions of most non-parametric tests. There are also variants of all classes of models mentioned here that can take violations of assumption 3 into account (e.g., generalised linear mixed models, GLMMs, Sect. 9.8).

Some data points will lie above and below the best-fit line, yielding positive and negative residuals respectively. When summing the residuals for a given candidate best fit-line, it is 'inconvenient' that there is a mixture of positive and negative residuals because they tend to cancel each other out. However, if they are first squared this turns them all into positive values. It turns out that the sum of squared (SS) residuals has some useful analytical properties and the best-fit line is thus found by minimising the SS between the sample data and the best-fit line. Thus standard regression is known as applying the least-squares (LS) technique.

9.3.2 Coefficient of Determination (r^2)

An impression of how close or 'tight' the observed data points are to the best fit line can be obtained by dividing the sums of squared residuals from the best-fit line by the sums of squared residuals from the overall sample mean, i.e., a horizontal line with its intercept (c), equal to the mean value of y. The value calculated is a sample statistic called the coefficient of determination (r^2, an estimate of the underlying population R^2) and is a proportion between 0 and 1. Thus, $r^2 = 1.0$ would indicate that all data points fall exactly on the best-fit line, so that in our sample data, all variation in y would be completely explained by x. As r^2 falls, the amount of variation in y that is explained by x decreases. It is possible to have two data sets described with the same best-fit line but with quite different values of r^2. The data set with the smaller r^2 would look more scattered when plotted on a graph (Fig. 9.4).

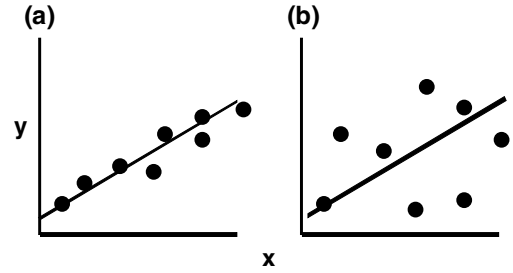


Fig. 9.4 Describing the 'tightness' of fit. These sets of sample data are described by the same best-fit line but have very different coefficients of determination (r^2). In **a** the r^2 is close to 1.0 while in **b** r^2 is closer to 0.0. We can say that a greater proportion of the variability in y is explained by x in (**a**) than in (**b**)

9.3.3 Significance in Regression

So far, we have conceptualised the least-squares, LS, approach to finding the best-fit line and described the tightness of data about this line, but we have not assessed the statistical significance of the patterns we see. Why might we want to do this when we have already described aspects of the relationship? Imagine an extreme, and deliberately trivial, case in which we have a set of data consisting of only two observations as shown in Fig. 9.5a: there is a positive slope (m) and $r^2 = 1.0$ because both data points lie directly on the best-fit line. Are you persuaded that such a relationship is genuine, and thus 'important'? Hopefully not, as the sample size

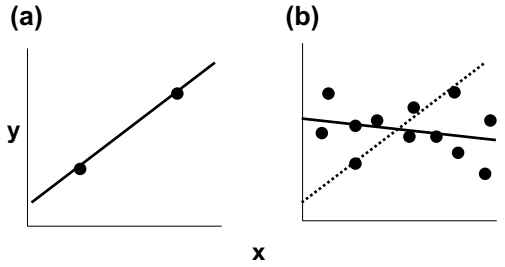


Fig. 9.5 How a relationship could occur by chance: the need for adequate sample sizes. **a** Best-fit line for just two data points yields a positive slope. However, adding more samples yields a very different picture (**b**) and it actually now appears that the underlying relationship may be negative

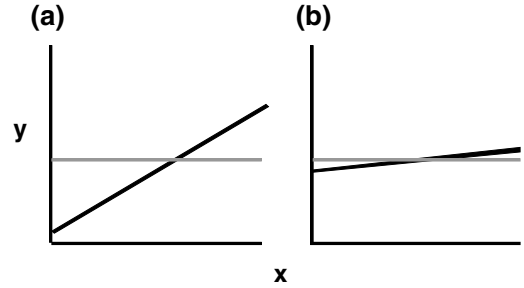


Fig. 9.6 Illustration of the need for significance tests to assess whether observed slopes differ significantly from zero. **a** The slope (upwards-sloping line) is steep and plausibly significantly different from zero (the horizontal line representing a hypothetical slope of zero in the underlying population, with its intercept being the mean value of y). **b** It is less clear whether the slope is significantly different from zero, hence the need for a formal criterion provided by regression analysis. Note that when performed mathematically, regression calculations incorporate information about the coefficient of determination (r^2) and sample size (n). Thus, with a higher r^2 and greater n, a given slope is more likely to be significant

(and hence power) is too small to perform any meaningful analysis. If we collected more data and added it to the plot, the best-fit line would almost certainly change and might even slope in a different direction (Fig. 9.5b).

Now that we have a larger set of data, do you think there is a positive (upward-sloping) relationship between x and y? Or maybe a negative (downward-sloping) one? Or perhaps there is no important relationship at all (i.e., the underlying 'slope' in the population is flat)? Standard regression addresses these questions by asking whether any relationship (i.e., positive or negative slope) between the variables is significantly different from zero (perfectly horizontal). Imagine you had collected a set of data and obtained a fairly steep slope (Fig. 9.6a). From this information alone it is plausible that the relationship will turn out to be significantly different from $m = 0$ (though this will depend on sample size and the degree of scatter). But what about Fig. 9.6b? The slope is not very steep, but it is not zero. Clearly, we need a formal criterion to help us decide whether the slope of the best-fit line is significantly different from zero. The formal output from regression follows the same format as the output from ANOVA and is dealt with in Sect. 9.4.3; let us first consider a key concept behind deciding between candidate descriptions of patterns in our data: parsimony.

9.3.4 Parsimony in Regression

One way to think about hypothesis tests of regression coefficients is asking whether the information gained about the data (e.g., the slope) is worth the 'cost' of including it in the description of patterns in the data. Including the slope into a description of patterns in the data makes the description more complex and possibly considerably, but at least slightly, more accurate. The best-fit line can be expressed as:

$$y = mx + c \tag{9.2}$$

This equation (or 'model' of our data) contains two parameters which are estimated from the data: the intercept (c), and the slope (m) (Fig. 9.7). If we were to conclude that the slope of the best-fit line is not significantly different from zero, we are effectively saying that data can be adequately described as the sample mean of y (Fig. 9.8). If $m = 0$ then $mx = 0$. This implies that our two-parameter model (Eq. 9.2) can be replaced by a one-parameter model:

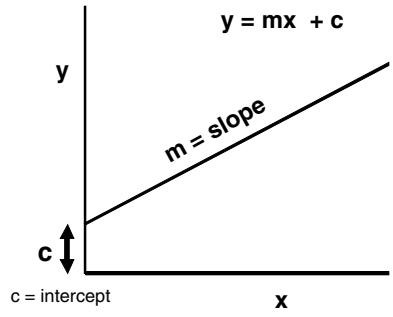


Fig. 9.7 The equation for a straight line ($y = mx + c$) is comprised of two variables and two parameters. x and y represent the explanatory and response variables respectively; m and c are parameters, the slope and intercept respectively

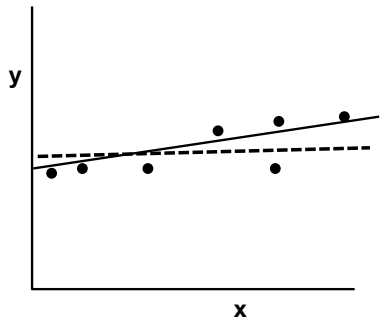


Fig. 9.8 Principle of parsimony in regression. Standard regression is essentially asking 'is the best-fit line (solid line with equation: $y = mx + c$) the simplest adequate description of the data, or would we be better served by simply using the mean value of y (dashed line with the equation: $y = c$)?'

$$y = c \tag{9.3}$$

which contains one less parameter and is thus a simpler, yet still adequate (i.e., more parsimonious), description of the data.

9.4 Analysis of Variance (ANOVA)

9.4.1 The Regression–Analysis of Variance Continuum

Imagine that we want to design an experiment to evaluate whether light intensity affects plant growth. We have chosen this non-entomological example because virtually everyone already knows that plants tend to grow more when there is more light available to them, and we want to focus attention on issues of design and analysis rather than on the results of our imaginary experiments. We have a long laboratory bench with a dark–light continuum (i.e., there is no light at one end of the bench and bright light at the other) and twelve plant pots each containing one seedling. We will put the pots on the bench and measure the growth of each seedling one week later. For the sake of simplicity, we consider that the plants respond to the environmental conditions independently of each other (assumption 3, Box 9.1).

Now, we need to decide how to arrange the pots on the bench. One possible design would be to spread the plants evenly along the bench. In this case we will obtain one measure of growth for plants at each of twelve (perfectly known; see assumption 1, Box 9.1) intensities of light. To assess whether growth is affected by light intensity we can carry out a regression analysis asking 'Is growth associated with light intensity', i.e., is the slope significantly different from zero? Light intensity is being treated as a continuous explanatory variable (Fig. 9.9) and all replicates are considered as independent (assumption 3 in Box 9.1).

An alternative design would be to place six plants at the dark end of the bench and six at the bright end. If we do this, we have two groups of plants ('dark-condition' plants and 'bright-condition plants'), and light, the single explanatory variable, has two categories (levels). Light intensity is now being treated as a categorical variable. To visualise the effect of categories of light intensity on growth we can construct a bar chart, with the heights of the bars representing the sample mean growth of each of the two groups of plants (Fig. 9.10). We may also want to show uncertainty in our estimate of the underlying population means using vertical lines extending from the tops of the bars to the lower and upper bounds of the 95% confidence interval (Fig. 9.10).

To test statistically whether growth is affected by light intensity we need to ask 'Is the growth of

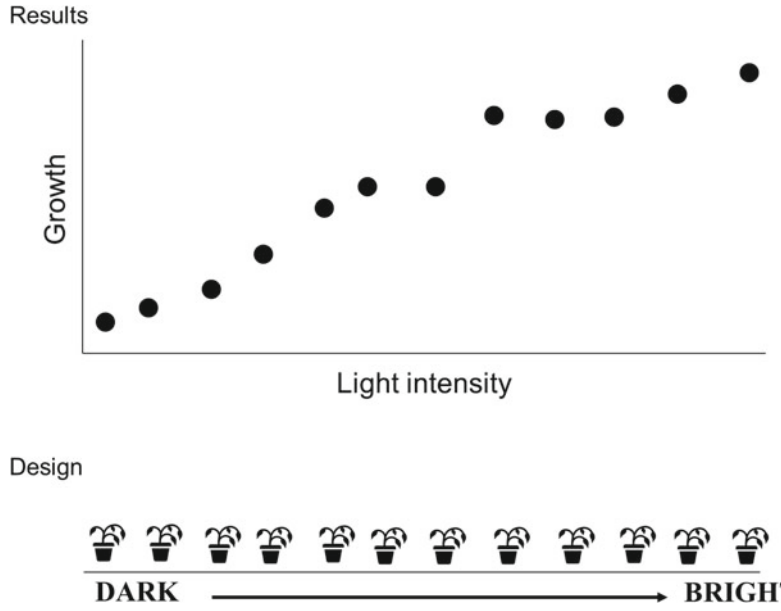


Fig. 9.9 A regression-oriented design. Twelve plant pots are spread evenly along the dark–light continuum and regression analysis is used to assess the whether the slope estimated from the sample data is significantly different from zero

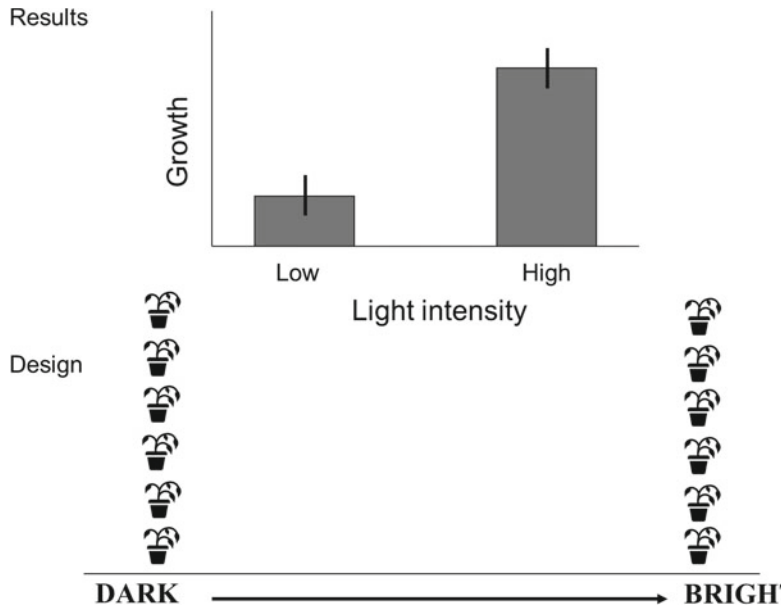


Fig. 9.10 An ANOVA-oriented design. The twelve plant pots are placed into two groups of six, and ANOVA is used to assess the difference in mean growth between the groups. Vertical lines represent schematically represent the 95% confidence intervals

the dark-condition group of plants significantly different from the growth of the light-condition group of plants?' In other words 'Is the difference in sample means greater than what we would expect due to chance alone, assuming the underlying population means are equal?' A method that can address this is called analysis of variance (ANOVA). In the case of assessing differences between two means a two-sample *t*-test can equivalently be used, but this is just a special case of an ANOVA.

Another design involving groups of plants would be to place four plants at the dark end of the bench, four in the middle and four at the bright end (Fig. 9.11). Now the explanatory variable, light intensity, has three levels, but ANOVA can still be carried out to assess the differences between the groups in much the same

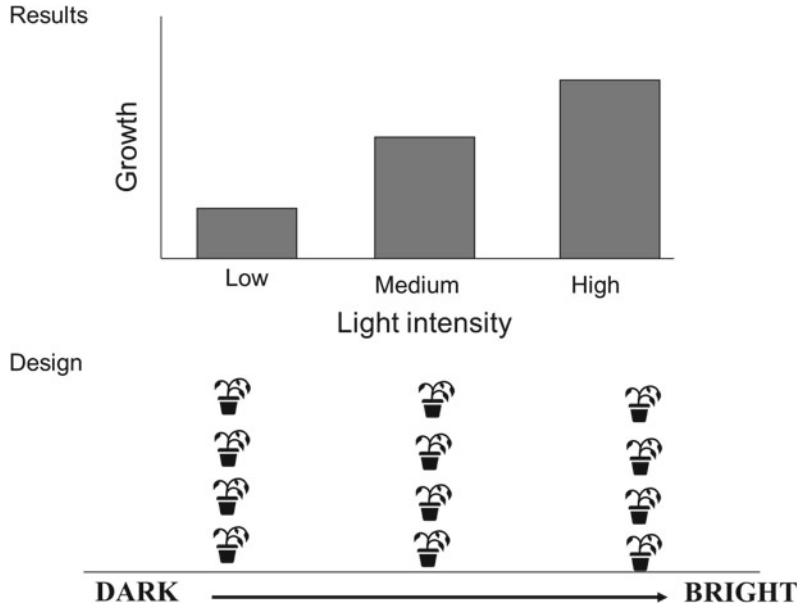


Fig. 9.11 An ANOVA-oriented design. The twelve pots are placed into three groups of four. Analysis of variance can assess the difference in mean growth between the three groups. (We do not include error bars on this figure or others in this series, in order to retain focus on the key purpose of these illustrations, but error bars should normally be included)

way as when there were only two groups of plants. Of course, we could take this a step further and arrange the plants into four groups of three plants along the bench (Fig. 9.12a) or even six groups of two plants (Fig. 9.12b) and still use ANOVA. The next logical step, 12 'groups' of one (Fig. 9.12c) would bring us back to where we started, that is the regression design, represented by a scatter plot rather than a bar chart (Figs. 9.9 and 9.12c are effectively the same).

It should now be obvious that there is no clear distinction between regression and ANOVA. They are simply at opposite ends of a continuum. At one end of the continuum (regression) the explanatory variable is continuous and at the other end (ANOVA) it is categorised in the most extreme way (just two treatment levels). Although beyond the scope of this introductory chapter, it can be shown that ANOVA is a special case of a multiple regression model with the categorical variable expanded to a series of dummy variables.

9.4.2 How ANOVA Works

Standard ANOVA can be used to explore differences (effects) in the means of the response variable between two or more groups within the explanatory variable. The assumptions of ANOVA are exactly the same as those for regression (Box 9.1). ANOVA is most often visually represented by bar charts, with the response variable on the *y*-axis. As with regression, inspecting plots of the data and detecting patterns is a good way to start the process of interpretation and analysis. We then might want to assess the significance of any differences between the means observed in our sample data.

ANOVA basically asks whether a difference as large as the one observed might be expected due to chance alone, assuming the underlying population means in all groups are equal. Imagine you had collected a set of plant growth data and obtained a fairly large difference in sample mean plant growth between the two light intensity groups, as illustrated in Fig. 9.13a. From this information it seems plausible that the observed difference will turn out to be significant (in reality, we would also want to have information on the size of the data set and the degree of variation around each of the means, analogous to discussion in Sect. 9.3.3). However, what if you obtained the result illustrated in Fig. 9.13b? There is a difference in mean growth between the two groups, but it is small. As with regression

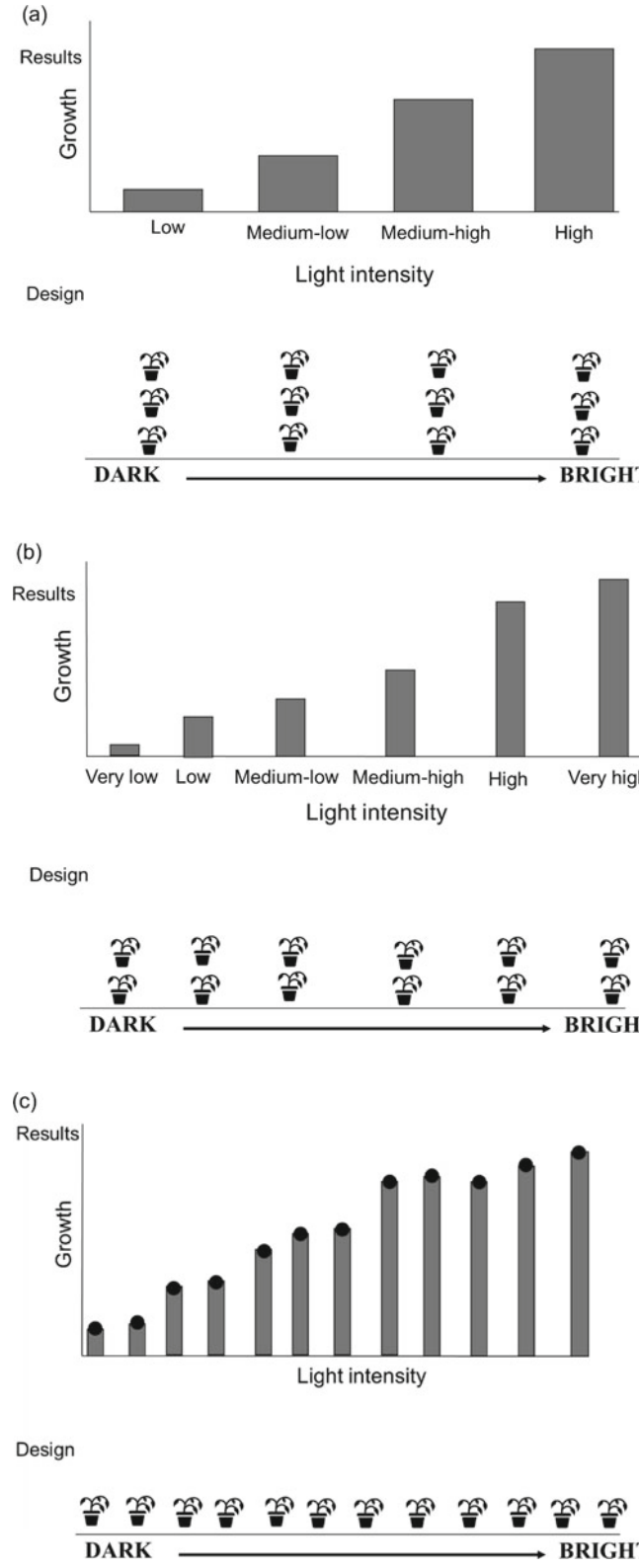


Fig. 9.12 Intermediate experimental designs and back to regression

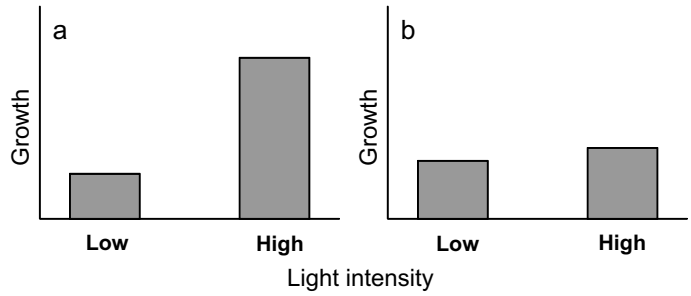


Fig. 9.13 Illustration of the need for significance tests to assess whether observed differences in sample means are important. **a** The difference is quite likely to be significantly different. **b** It is less clear whether the difference is significantly different, hence the need for formal criteria provided by ANOVA (compare this figure to Fig. 9.6). Note that when performed mathematically, ANOVA calculations incorporate information about the coefficient of determination (r^2) and sample size (n). Thus, with a higher r^2 and n, a test for a given difference in population means is more likely to be significant. Note that we would usually show 95% confidence intervals around the means, which would provide information on the precision of our sample mean estimates

(Sect. 9.3.3), we need a formal criterion to help us decide whether a given difference is significantly different from zero. ANOVA provides this by asking whether the information that there are two groups is worth the 'cost' of this more complex description of the data. If a difference turns out to be non-significant, we can merge the data from the two groups together and describe plant growth by the overall sample mean (and its 95% confidence interval). This, again, is the principle of parsimony in action (Sect. 9.3.4).

Imagine that the result illustrated in Fig. 9.13b derives from sample data on the growth of 8 female and 8 male plants (this is probably a botanically unrealistic example, but this need not be of concern here). We can see that the sample mean growth of each group (category) of plants is different and we want to use ANOVA to assess whether a difference as large as that observed is likely to have arisen by us taking two random samples from populations that in fact had the same underlying population means. For the purpose of illustrating the method, it is useful to plot the data on a scatter graph in the order they were collected (here we follow Crawley's, 1993, explanation but of course we would not usually plot the data like this for presentation in a publication or a report), along with the overall sample mean value of plant growth, as shown in Fig. 9.14a.

Now, the growth of most plants is different from the overall sample mean. The vertical differences between each data point and the sample mean line are the residuals. The data and the associated residuals are illustrated in Fig. 9.14b. The residuals are shown by themselves in Fig. 9.14c. We can gain an impression of how well the overall sample mean fits (describes) the data by summing all 16 residuals. As with regression, we convert all residuals to positive numbers by squaring each value. The resulting sums of squares (SS) will be large if there is a large variation in plant growth and small if plants tend to grow by similar amounts.

So far, we have ignored the fact that there are two sexes of plants in our data set, despite evaluation of the consequences of sex for plant growth being the goal of our investigation. We now turn our attention to plant sex and calculate the sample means for male and female growth separately. The mean growth of male plants and the mean growth of female plants can then be plotted as two separate lines, each with associated residuals: those from male data points to the male mean and those from female data points to the female mean (Fig. 9.14d). To drive home the message that ANOVA works with residuals, the new set of residuals is shown in Fig. 9.14e.

To determine whether the set of residuals from the overall sample mean or the set of residuals

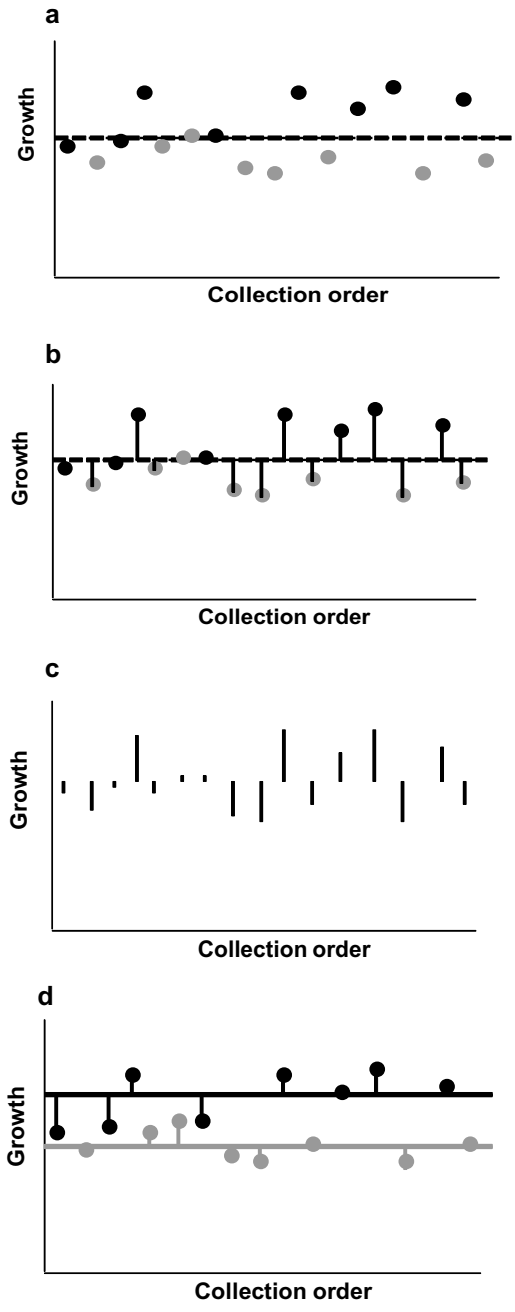


Fig. 9.14 **a** Growth values for male (black) and female (grey) plants in order of collection, together with the overall mean (dashed). **b** Positive and negative total residuals (vertical lines) between data points and the overall sample mean. **c** Total residuals by themselves. **d** Separate means and residuals for male and female plants. **e** Separate residuals for male and female plants by themselves. **f** Sum of total residuals (left) compared to sum of residuals from male and female plants (right). As in Fig. 9.2, we note that it is not standard practice to show residuals but we use them here to illustrate the statistical method

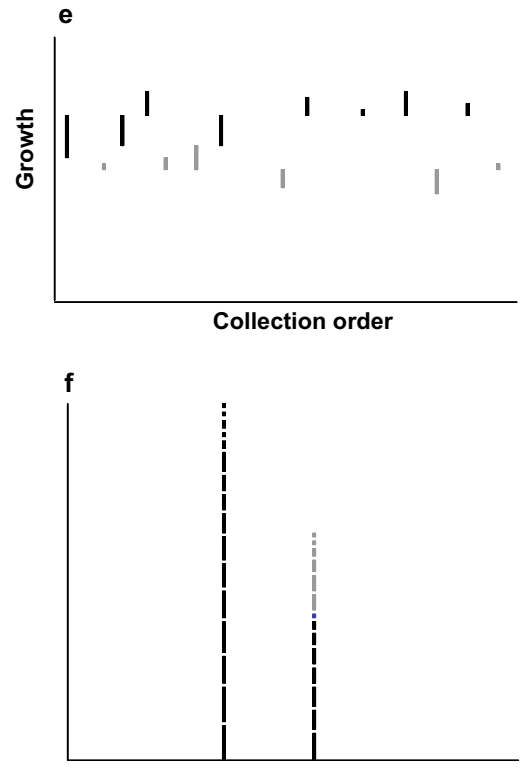


Fig. 9.14 (continued)

from the two separate sample means provides a better description of the data, we simply compare the summed residuals and see which set is smaller. To do this mathematically we need to work with sums of squares, SS, as described in Sect. 9.3.1. We can, again, visualise the process by imagining picking the two sets of residuals up and putting them into two piles (Fig. 9.14f). In this case, it is clear that the SS that takes plant sex into account is lower than the SS from the overall mean, and thus might provide a better description of the data. Like regression, ANOVA is a least-squares technique because we are finding the description of the data that minimises the SS. The improved description of the data (reduction in SS) has to be sufficient to warrant the 'cost' of estimating an additional parameter (taking plant sex into account rather than ignoring it). As described above (Sect. 9.3.3), one way we can approach this issue is to use a hypothesis test.

You should notice that the above description of ANOVA and the section on regression are

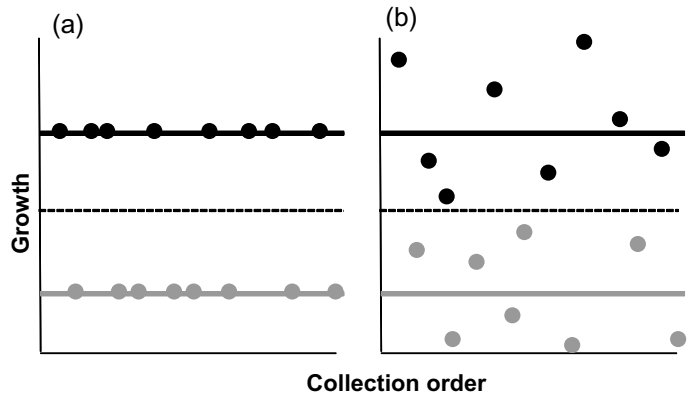


Fig. 9.15 Describing the degree of scatter/tightness in ANOVA. These sets of data are described by the same overall sample mean (dashed lines) and the same sex-specific sample means (solid lines), but have very different coefficients of determination (r^2). In panel **a** r^2 is close to 1 while in panel **b** it is closer to 0

very similar. The key difference is that regression works with residuals from a best-fit line, while ANOVA works with residuals from the sample means of the individual levels of the explanatory variable. As in regression, we can obtain an impression of how well our explanatory variable (in this case plant sex) explains variation in plant growth by dividing the SS from the male and female means by the total SS to obtain r^2. As before, r^2 close to 1.0 would indicate that all data points lie close to the sample mean values for each level of the explanatory variable (Fig. 9.15 a) and a value close to 0.0 would indicate that data points lie further from these means (Fig. 9.15b). It is worth comparing Fig. 9.15 to the equivalent illustration for regression (Fig. 9.4).

9.4.3 Output from Regression or ANOVA

When we carry out regression or ANOVA in statistical software, the results are output in a standard format. One such output is the ANOVA table (Table 9.2), a name that is perhaps misleading as ANOVA-type tables can be output for any GLM. The test statistic is known as the F ratio, or simply F named after R. A. Fisher (Sect. 9.2). F has a known distribution under H_0 for given degrees of freedom. If the calculated F is greater than the critical F value for 5%, then there is a 5% or smaller probability of the observed slope (regression) or difference (ANOVA) occurring in the sample data due to chance under H_0, i.e., $P < 0.05$. A given slope or difference is more likely to be statistically significant if there is a larger sample size (n) (e.g., Taborsky, 2010) and there is a smaller degree of scatter, i.e., large r^2.

9.5 Analyses with Multiple Explanatory Variables

So far, we have considered the influence of a single explanatory variable on a response variable. The explanatory variable was either continuous (regression) or categorical (ANOVA). But what if we want to model the effect of multiple explanatory variables on a response variable simultaneously? This leads us to three new analyses each of which are, at least conceptually, extensions of the regression-ANOVA approach.

Analysis of Covariance (ANCOVA) tests the effect of two or more explanatory variables (at least one continuous and at least one categorical)

Table 9.2 Statistical output (ANOVA table) from regression analysis. Regression works by recognising three sources of variation, namely 'regression', 'error' and 'total'. The 'total sum of squares' (SS) is equal to the sum of the 'regression sum of squares' and the 'error sum of squares' (the latter is also known as 'residual sum of squares'). The mean squares (= sample variances) for the regression and error are calculated by dividing the respective SS by their degrees of freedom. The *F* ratio is calculated by dividing the regression mean squares by the error mean squares. The *F* ratio is then compared to critical values at the appropriate degrees of freedom to obtain the *P*-value. Note that the term 'error' is used in place of 'residual' (Sects. 9.2.5 and 9.3.1) here to avoid confusion with regression when abbreviations are used. Statistical output and calculations from ANOVA are arranged in exactly the same way, the only difference is that the source will be termed 'ANOVA' as opposed to 'regression' (and the ANOVA degrees of freedom may be >1)

Source	Sum of squares	Degrees of freedom	Mean squares	*F* ratio	*P*-value
Regression	Regression sum of squares (SSR)	1	SSR/1 = SSR	SSR/MSE	Software/statistical tables
Error	Error sum of squares (SSE)	$n - 2$	SSE/($n - 2$) = mean square error (MSE)		
Total	Total sum of squares (SST)	$n - 1$			

on a continuous response variable. If we want to test hypotheses, ANCOVA can test the significance of: (1) the slope(s) just like regression; (2) the between-group difference(s) in the response variable, just like ANOVA; and (3) whether the slope(s) differ(s) between groups, known as the interaction (Fig. 9.16). The interaction term often provides very useful information that is not available from carrying out separate regressions or ANOVAs on the sample data. This is one of the major advantages to using the parametric approach rather than a non-parametric approach: non-parametric tests do not readily combine and do not usually allow the assessment of interaction effects.

Factorial ANOVA is very similar to ANCOVA, the difference being that ANOVA deals with multiple categorical variables while ANCOVA deals with a mixture of categorical and continuous variables (Fig. 9.17). Factorial ANOVA might be thought of as two or more ANOVAs being carried out at the same time and is thus also known as 2-way ANOVA, 3-way ANOVA etc., as appropriate. Assuming an adequate sample size, considering more than one factor within the same analysis tends to reduce the residual sum of squares (Table 9.2) which consequently increases the *F* value and thus the power of the tests, though inclusion should be based on subject matter knowledge as well as other considerations, such as confounding variables. As with ANCOVA, factorial ANOVA can allow assessment of interaction terms which is not possible when each of the explanatory variables is assessed by separate 1-way ANOVAs, and thus an advantage of factorial ANOVA over a series of separate 1-way ANOVAs is that more information can be obtained from the sample data. However, interaction terms can be estimated and tested only if there is more than one replicate of each possible combination of the factors considered.

Multiple regression can simultaneously analyse the effect of several continuous explanatory variables on a response variable (Figs. 9.18 and 9.19). It models the slopes of each explanatory variable, just like regression, and can also assess for interaction effects by estimating an additional parameter to allow slopes of different magnitude (and possibly direction) when other explanatory variables vary. Such assessment of interactions often leads to a better understanding of patterns in the data than would be obtained by a series of separate regression analyses or by failing to model interaction effects. In computational terms, multiple regression is essentially the same as ANCOVA and factorial ANOVA, but visualisation requires at least three dimensions.

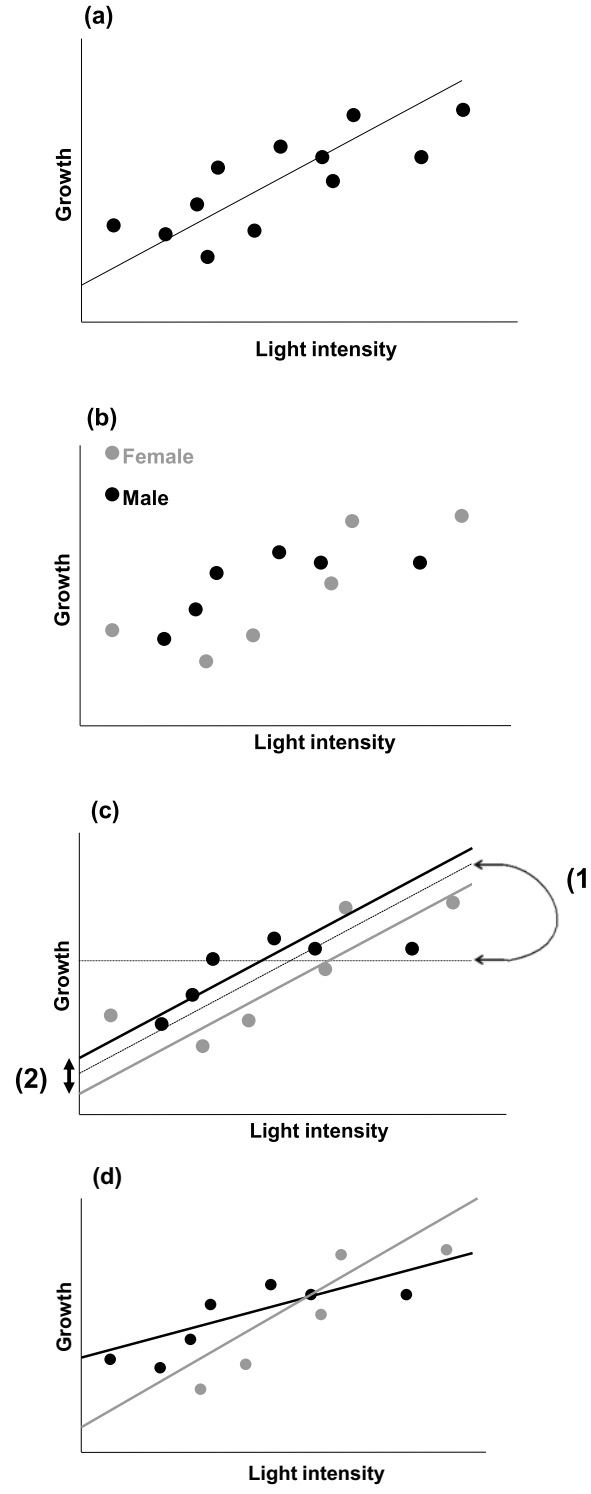


Fig. 9.16 Understanding analysis of covariance (ANCOVA). **a** The overall set of data with associated best-fit (regression) line summarising the effect of light intensity on plant growth. **b** Growth measurements have been made on two sexes of plant. **c** Two questions within ANCOVA: (1) does the overall slope differ significantly from zero? (2) do the intercepts (vertical distances between the lines at $x = 0$) differ significantly from each other? **d** A third question that can be asked within ANCOVA: are the slopes of the two lines significantly different from each other?

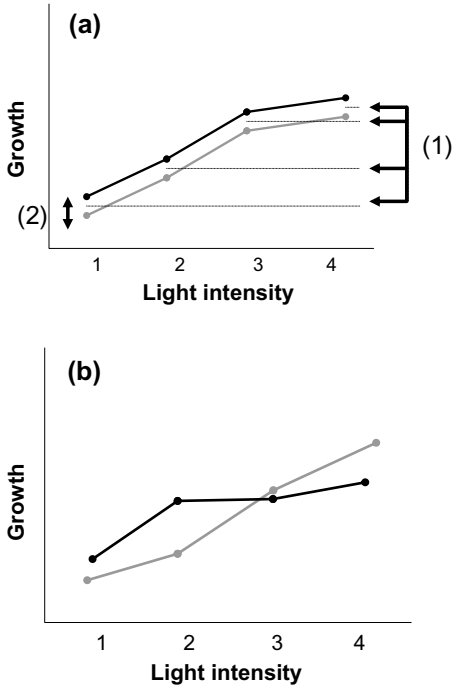


Fig. 9.17 Understanding factorial ANOVA. On each graph a dot indicates the sample mean growth for a given level of each explanatory variable (factor) and the lines simply join the dots to illustrate the trend. Panel **a** illustrates the questions: (1) 'are the means for plant growth at the four light intensities significantly different?' and (2) 'are the means of male and female plant growth significantly different?' Panel **b** illustrates an interaction between light intensity and sex (parallel lines would indicate no interaction). As with other illustrative figures, to retain focus on the key messages we do not show error bars

Accordingly, the results of multiple regression can be difficult to present graphically if several explanatory variables have significant effects on the variable to be explained. One solution is the partial effect plot, which shows predictions (line of best fit and confidence bands) for each explanatory variable of interest while holding the other predictors constant at their average (Harrell, 2015). Since these methods can also analyse the interaction between explanatory variables in multiple regression, ANCOVA and factorial ANOVA, information on interactions can also be included in the standard output, but otherwise it looks similar to that of regression or ANOVA (Table 9.3).

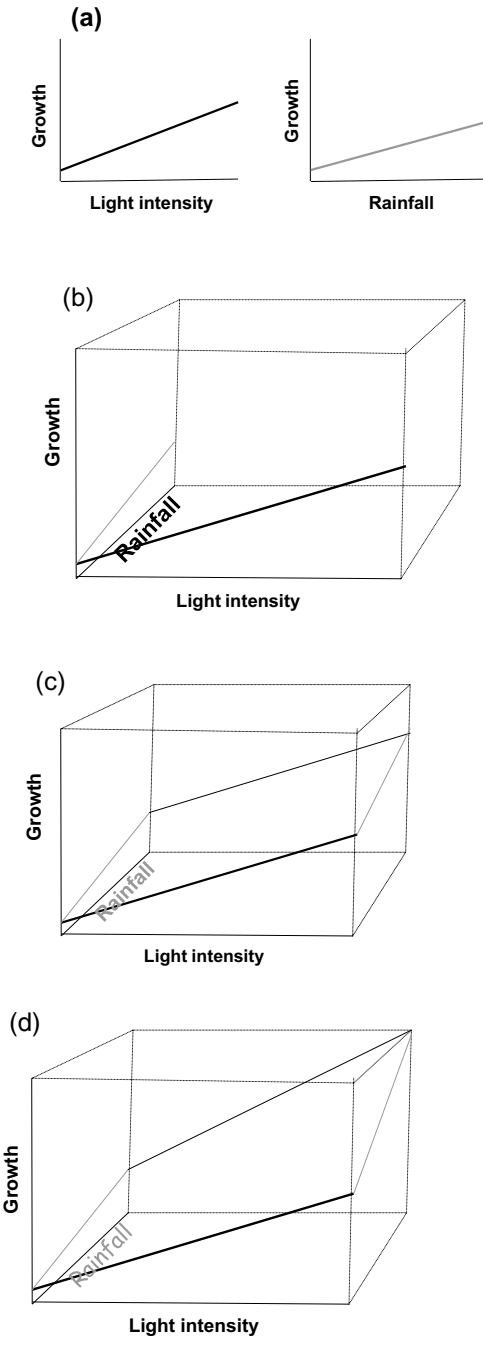


Fig. 9.18 Understanding multiple regression: two positive regression relationships with and without interaction. **a** The effect of light intensity and rainfall on growth is shown on two separate graphs. **b** A third axis, *z*, is introduced to enable plotting of two continuous explanatory variables on a single graph. **c** Flat plane indicates no interaction between explanatory variables. **d** Twisted plane indicates interaction between explanatory variables

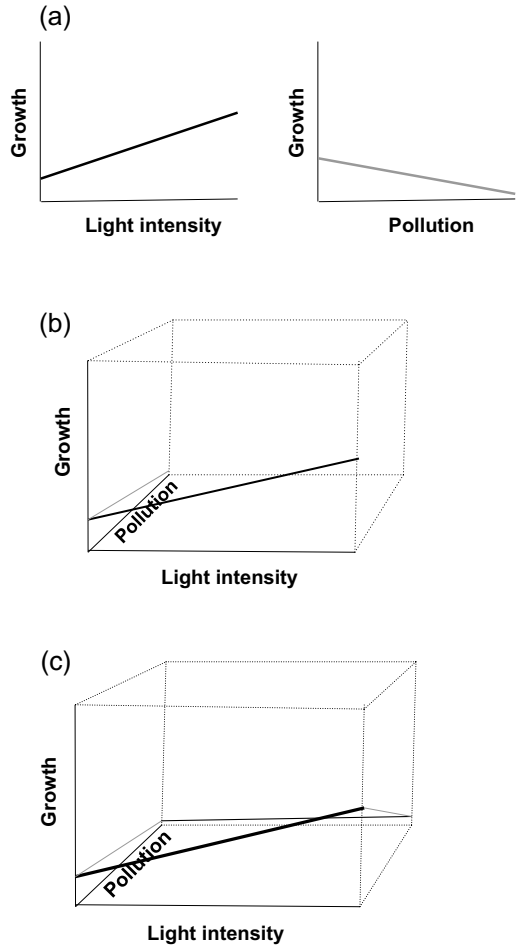


Fig. 9.19 Understanding multiple regression: a positive and a negative regression relationship with interaction. **a** The effect of light intensity and pollution on growth is shown on two separate graphs. **b** A third axis, z, is introduced to enable plotting of two continuous explanatory variables on a single graph. **c** Twisted plane indicates interaction between explanatory variables

9.6 Model Simplification

We have now dealt with a number of statistical methods to explore the effect of single (regression, ANOVA) or several (multiple regression, ANCOVA, factorial ANOVA) explanatory variables on a continuous response (dependant) variable. In principle, a large number of explanatory variables (limited by the overall number of replicates) can be used to explain variation in the response variable. In practice, we should avoid using more explanatory variables than can be supported by the sample size since this may lead to overfitting, which arises when the statistical model begins to describe 'noise' in the sample data rather than effects in the underlying population. Furthermore, it is best to focus on explanatory variables that are likely to be of biological interest and, if analysing experimental data, the variables that the experiment was designed to test. One valid modelling strategy that side-steps the issue of model simplification is to pre-specify a candidate set of explanatory variables. The number of explanatory variables to be modelled should be carefully selected based on subject matter knowledge (e.g., interactions, collinear terms) and the number of terms which can be modelled for the given sample size (for guidelines see e.g., Harrell, 2015). In this section we will consider methods of obtaining a parsimonious model of the data when there are multiple candidate explanatory variables. We note that this issue is the subject of considerable debate and that options and recommendations are evolving.

Imagine we wish to set up a general linear model to test the effect of four explanatory variables x_1, x_2, x_3, and x_4 on a single dependant variable y. We first consider procedures that involve adding and/or eliminating explanatory variables in sequence (Sect. 9.6.1), which usefully illustrate the concept of obtaining parsimony, and then briefly discuss alternative approaches to model simplification or selection (Sect. 9.6.2).

9.6.1 Top-Down or Bottom-Up?

A top-down approach is to start by fitting all candidate explanatory variables and their interactions to the model, the so-called maximal model which contains main effects (i.e., the four explanatory variables) and their various

Table 9.3 Statistical output for multiple regression, factorial ANOVA or ANCOVA. The arrangement is much the same as for standard regression or ANOVA (i.e., ANOVA table) but we now have two explanatory variables (A and B) and also their interaction, creating two additional rows compared to Table 9.2. Factor A has r levels and factor B has c levels. If an explanatory variable is continuous, then its degrees of freedom will be 1. The resulting change in the partitioning of variance changes to calculations in the 'error' and 'total rows' compared to Table 9.2

Source	Sum of squares	Degrees of freedom	Mean squares	F ratio	P-value
Variable A	SSA	$r - 1$	MSA	MSA/S^2	Software/statistical tables
Variable B	SSB	$c - 1$	MSB	MSB/S^2	Software/statistical tables
Interaction A*B	SSAB	$(r - 1)(c - 1)$	MSAB	$MSAB/S^2$	Software/statistical tables
Error	SSE	$rc(n - 1)$	$S^2 = SSE/[rc(n - 1)]$		
Total	SST	$rcn - 1$			

interactions, which will be of the first, second and third order, i.e., interactions between two, three and all four main effects (Fig. 9.20). We are not obliged to consider all levels of interaction in the maximal model. For instance, we may elect to initially include only main effects and first-order interactions. The choice could be influenced by the overall size of the data set and the numbers of main effects (and the numbers of factorial levels) considered. Once terms of interest have been included in the maximal model, statistical software packages will output test statistics and P-values for each main effect and their interactions. From this stage there are numerous possible ways to proceed towards a more parsimonious model (Crawley, 1993, 2002; Quinn & Keough, 2002; Mundry & Nunn, 2009). One approach for model simplification is to start with the highest-order interaction and work backwards in a stepwise manner (Table 9.4) (e.g. Charrat et al., 2023). Many statistical software packages (e.g., GLIM, GenStat, R) allow one to easily drop terms from a model and assess the significance of the effect using an F test and associated P-value.

Assume our maximal model is that shown in Fig. 9.20 and that we adopt the conventional $\alpha = 0.05$. We first test the effect of dropping the highest-order interaction from the model. If the change is significant ($P < 0.05$) we add this term back to the model. Because all other fitted variables are involved with the highest-order interaction term, and we cannot simplify the model further by reducing the number of explanatory variables, we have thus achieved the minimal adequate model (unless we wish to consider aggregating some factor levels of the categorical variables, Sect. 9.6.3). However, if dropping the third-order interaction does not result in a significant change ($P > 0.05$), we would not add this interaction term back to our model and would focus next on the second-order interactions.

We would select one of the second-order interactions (e.g., the one which looks the least likely to be significant on the basis of the parameter estimates of the current model), and formally test its significance by dropping it from the model. If $P \leq 0.05$, we would add it back into the model or, if $P > 0.05$, we would leave it out of the model. After all the second-order interactions have been dropped and tested, we can then go to the first-order interactions and repeat the process until we reach the main effects.

Main effects are tested in the same way as above, i.e., dropped one by one from the model

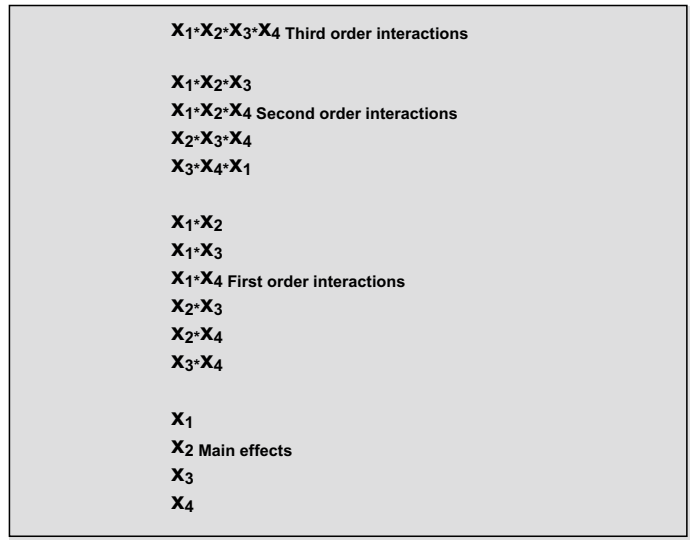


Fig. 9.20 Main effects and interactions (denoted by *) of the first, second and third order for four explanatory variables x_1, x_2, x_3 and x_4. The most complex possible maximal model is the model containing all variables and all possible interactions, but note that we may choose to adopt a simpler maximal model, such as one containing only main effects and first-order interactions

Table 9.4 Model simplification: terminology and steps involved in top-down analyses (after Crawley, 1993). A 'saturated (full) model' has perfect fit; zero deviance, zero degrees of freedom and one parameter for each observation (in some model types, deviance, Sect. 9.7.1, is the same as sums of squares, Sect. 9.3.1). A 'maximal model' contains all factors, interaction terms and covariates under consideration. A 'current model' contains a number of parameters less than or equal to the maximal and greater than or equal to the minimal model. A 'minimal model' contains the minimal number of terms to adequately describe patterns in the data, in which all parameters are significantly different from zero, and no important terms have been omitted. A 'null model' contains only one parameter, the grand mean, and the deviance is equal to the total sum of squares in Gaussian models. The following sequence of steps in stepwise model simplification provides a general guide

Step	Procedure	Explanation
1	Fit the maximal model	Fit all the factors, interactions and covariates of interest. Note the residual deviance (or SS) If you are using Poisson or binomial errors, check for overdispersion and rescale if necessary
2	Begin model simplification	Inspect the parameter estimates Remove the least significant terms first, starting with the highest-order interactions, progressing on to lower-order interaction terms and then main effects
3	If the deletion causes an insignificant increase in deviance	Leave that term out of the model Inspect the parameter values again Remove the least significant term remaining
4	If the deletion causes a significant increase in deviance	Put the term back in the model These are the statistically significant terms as assessed by deletion from the maximal model
5	Keep removing terms from the model	Repeat steps 3 or 4 until the model contains nothing but significant terms This is the minimal adequate model If none of the parameters are significant, then the minimal adequate model is the null model

to assess the significance of the change, and not replaced in the model if $P > 0.05$. At the end of this process we are then left with the minimal adequate model, which is a parsimonious description of relationships in the data and the major goal of the analysis (Table 9.4).

An alternative approach is to build the model in a forwards bottom-up manner. As one might expect, this approach starts by testing the significance of adding a series of individual main effects into the model (immediately removing them if they are not significant) and then proceeding to add first-, then second-, then higher-order interactions. One drawback of the bottom-up approach is that a variable may not be significant on its own but might contribute significantly via higher-order interactions. A further drawback is that there is no formal means of deciding which order to enter terms into the model (in contrast to the top-down analysis where the decision can be based on the parameter estimates in a current model).

While we advise using the top-down rather than bottom-up approach where possible, we do note that there is concern in the statistical literature that backwards elimination procedures are not always able to select the most influential variables from an initial set. For instance, dropping a term during a top-down procedure might result in a previously dropped and excluded factor becoming significant, or a previously dropped and re-included term becoming non-significant. One procedure to deal with this would be to re-restart the analysis each time a term is dropped or added in a combined top-down and bottom-up manner (Wajnberg et al., 1999).

9.6.2 Beyond Forwards or Backwards Procedures

The decision to include or exclude terms in backwards or forwards stepwise procedures is based on some measure of the additional change in residual variance accounted for by the relevant variable (Roff, 2006), and the procedures outlined above effectively involve a series of null-hypothesis significance tests (e.g., F ratio tests). Stepwise methods should be used with caution. In particular, treating confidence intervals and P-values at the end of an automated selection algorithm in the same way as those derived from a pre-specified model is invalid. Some software packages allow estimates from such stepwise methods to be 'corrected' using bootstrap routines (e.g., in the R package 'rms'). An alternative approach is to use Akaike's information criterion (AIC) which adjusts (penalises) the deviance (Sect. 9.7.1) for a given model by the number of predictor variables modelled (Quinn & Keough, 2002; Holmes et al., 2023). A low AIC implies better model fit and if a number of models have similar AIC values the model with the fewest terms should be selected (Quinn & Keough, 2002). Note, however, that employing AIC to choose a parsimonious model does not in itself involve tests of null hypotheses. While this may take some getting used to for many biologists, there are compelling arguments for reducing the current emphasis on null-hypothesis significance testing (Nakagawa & Cuthill, 2007; Halsey, 2019, Sect. 9.8.1).

9.6.3 Differences Between Factor Levels

We have so far looked at detecting significant differences in means between multiple groups or factor levels (ANOVA, ANCOVA, factorial ANOVA), but this alone will not inform us of where the significance actually lies. Supposing we perform ANOVA for three levels A, B and C of a single categorical explanatory variable (i.e., a factor). If we obtain $P < 0.05$, we know there is a significant difference between means of at least two levels of the factor, but we do not know which ones. In fact, the model is assessing whether each sample originated from a population with the same mean. It could be because A is different from B and/or C, or B is different from

C or because all three levels are different from each other. One possibility would simply be to perform three separate ANOVA tests, though this is unwise. Recall that every time we carry out a statistical hypothesis test we are accepting a 5% probability of Type I error (Sect. 9.2.4), so the more tests we perform the greater the likelihood of obtaining a falsely significant result (McDonald, 2014, provides clear guidance on multiple hypothesis testing and how to correct for it).

One approach is to aggregate factor levels. Thus, in our above example we could aggregate *A* and *B* into a new, common, level '*AB*' and test the significance of the change using an *F* test. Note that *C* is present in both versions of the model so the test using aggregation is not quite the same as a separate test of the difference between *A* and *B*. If $P > 0.05$ we would retain the aggregation; this is essentially saying that there is no significant difference between *A* and *B* and we are better served by using the mean of *A* and *B* combined. Conversely, if $P \leq 0.05$, this implies a significant change in the model and so we would discard the aggregation and return to using our original groups *A* and *B* (i.e., there is a significant difference between *A* and *B*). Aggregation of factor levels can also be used in situations where other continuous (ANCOVA) or categorical (factorial ANOVA) explanatory variables have a significant interaction with the categorical variable for which factor levels are being aggregated.

An appealing aspect of aggregating factor levels is that the procedure remains within the framework of model simplification we have already seen (Sect. 9.6.1). However, there are other approaches to comparing factor levels that are commonly used. One simple way to test for significant differences between means of different factor levels is, for example, to use Tukey's honestly significant difference (HSD) test, which compares each group mean with every other group mean in a pair-wise manner and controls the family-wise Type I error rate to no more than the nominal level (e.g., 0.05) (Quinn & Keough, 2002).

9.7 Generalised Linear Models

We have now built up an understanding of standard parametric methods, i.e., regression, multiple regression, ANOVA, factorial ANOVA and ANCOVA: these are collectively known as general linear models (e.g., Grafen & Hails, 2002) which all use the same set of assumptions, including that the errors are normally distributed (Box 9.1). However, the error distribution for some types of data is not likely to be normally distributed and thus the application of standard parametric methods for normally distributed data is no longer appropriate and likely to give misleading (incorrect) answers.

The traditional approach to non-normality is to transform the sample data prior to standard analysis in an attempt to 'normalise' it (i.e., make the data conform to the assumption). Rather than forcing the data to fit the assumption of normality, the modern approach is to adopt initial assumptions that are more likely to fit the data. The assumption of a normal distribution can be replaced by an assumption of another exponential-family distribution, i.e., the Poisson, binomial, negative binomial, Weibull, or gamma distributions (Sect. 9.2.5). This wider family of methods, which includes the sub-family of general linear models, is known as generalised linear models, or GLMs (sometimes called GLZs). Responses are modelled by inclusion of a 'link function' to connect the mean of the response variable to the linear combination of parameters estimated. Here we discuss GLMs that adopt the initial assumption that errors conform to either a Poisson distribution (e.g., log-linear models) or a binomial distribution (e.g., logistic regression). Other assumptions are the same as for general linear models (Box 9.1).

9.7.1 Log-Linear Models: Count Data

The response variable in many studies of natural-enemy biology will be gathered as counts, i.e., positive integers, with a lower bound of zero and

no upper bound. For instance, the number of eggs in a stink bug egg mass (Abram et al., 2023) or in a parasitoid's clutch (Goubault et al., 2007) or a parasitoid's lifetime fecundity (Zaviezo & Mills, 2000). When data consist of counts it is a reasonable starting point to assume that the errors are Poisson distributed. With counts of 'large-valued' numbers (e.g., 50, 82, 670) the mean will be large and the Poisson distribution tends to a normal distribution, but with counts of small value (e.g., between 1 and 6, and also with counts of zero), the Poisson distribution is quite different in form (Fig. 9.1).

To deal with non-normality the data could be, as mentioned above, transformed, e.g., by log-transformation prior to employing an analysis that assumes normally distributed errors, although such transformation does not necessarily achieve normality, especially when there are many (say, 20–30) observations of zero in the data set, and thus this approach is no longer generally advised (O'Hara & Kotze, 2010). A better alternative is to use an analysis that initially assumes that the errors are Poisson distributed (we revisit this initial assumption in Sect. 9.7.3). Such analyses are known as Poisson or log-linear models and there are log-linear versions of each of the general linear models we have previously discussed. Log-linear analyses work in essentially the same way as those standard analyses but, when assuming Poisson-distributed errors, estimates of parameters are performed using maximum likelihood (see below). Of the possible test statistics, so-called likelihood ratio tests are χ^2-distributed under the H_0 (see below).

We illustrate the approach using another non-entomological and imaginary example: a set of data on the numbers of fish caught (response variable) in locations with different amounts of available food (explanatory variable). Figure 9.21a depicts a standard regression analysis of such data, along with the naïve assumption of a normal distribution of errors at various points along the regression line. It can easily be seen that this approach can predict the possibility of negative value observations, which of course is nonsense: we cannot catch a negative number of fish. Explicitly, note that the tail of the normal distribution extends below zero within the range of food abundances and also that extrapolation of the regression line to the left of the data (which we might do if we wanted to know how many fish we might catch when food was even less abundant than in the most spartan of our sampling localities) would predict negative count values. (We, however, in passing, generally advise against extrapolation due to its likelihood of inaccuracy.) Standard methods also assume that

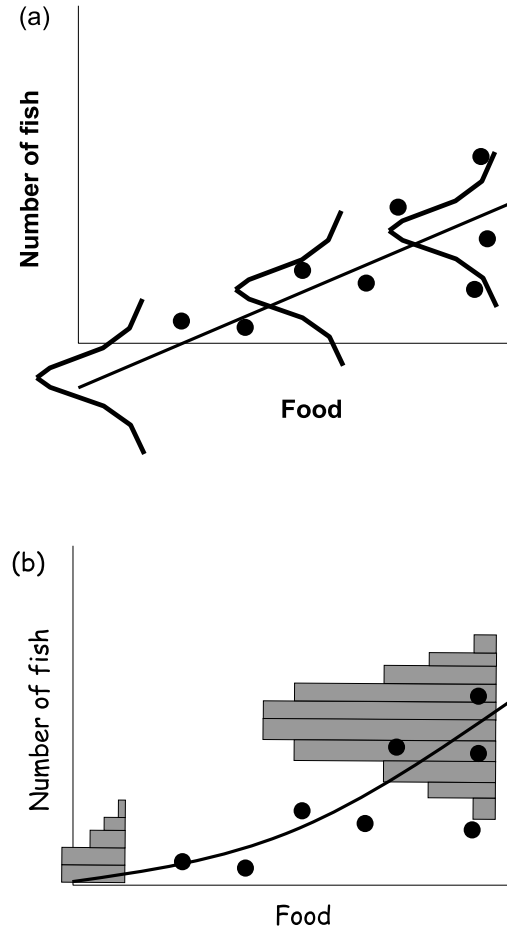


Fig. 9.21 Why the assumption of Poisson-distributed errors is appropriate for the analysis of count data. **a** A standard regression of Poisson-distributed data assumes impossible negative values and, incorrectly, constant variance. **b** These problems are solved using a log-linear regression: impossible negative data values are not assumed and the variance is correctly assumed to increase with the mean

the variance does not increase with the mean, while with count data it does. A potential solution to these problems is to use a log-linear regression that assumes Poisson-distributed errors (Fig. 9.21b). The Poisson distribution is a one-parameter distribution, specified by the population mean. In fact, in the Poisson distribution, the population mean equals the variance, therefore:

$$\frac{\text{variance}}{\text{mean}} = 1 \tag{9.4}$$

The regression equation for log-linear models is a simple modification of that for the straight line (Eq. 9.2): $\log(y) = mx + c$, such that the y value is now predicted by the antilogarithm of $mx + c$:

$$y = \text{antilog}(mx + c) \tag{9.5}$$

which can also be expressed as:

$$y = e^{(mx+c)} \tag{9.6}$$

Because the Poisson distribution lacks one tail at low mean values, impossible negative counts are never assumed. Neither are they predicted from extrapolation, as the regression line can never fall below zero (as the antilog of x is always positive or zero).

Unlike general linear models that use the least-squares (LS) technique for finding the best fit line, log-linear (and logistic models, Sect. 9.7.2) use maximum likelihood estimation (MLE). In non-technical language, this means that given the sample data and a particular model, the estimates of the parameters (i.e., slopes and the intercepts) are those that would make the observed data most likely. (Note that with normally distributed data, LS and ML are the same.)

When carrying out log-linear analyses assuming Poisson-distributed errors, significance is assessed using change in deviance based on MLE. Deviance has different mathematical definitions under different error structures: for normally distributed data, SS can also be called deviance but, with Poisson errors, deviances are not the same as sums of squares. The significance of a change in deviance ratio, G, is assessed using the χ^2 distribution at the appropriate degrees of freedom (rather than F ratio tests, Sect. 9.4.3, but note that G is asymptotically χ^2-distributed).

9.7.2 Logistic Analyses: Binary Data

The raw data in many studies of natural-enemy biology will be binary. Such data may give rise to proportions that range from zero to one. For instance, the proportion of eggs in a stink bug egg mass that were parasitised (Tillman et al., 2020; Cornelius et al., 2021) or the proportion of male parasitoids (i.e., the sex ratio) among the emerging progeny (Stahl et al., 2018; Holmes et al., 2023; Liu et al. 2023). For clarity, a proportion is calculated as the number of times something of interest did happen divided by the total number of times if might have happened, with the former (the numerator) thus never exceeding the latter (the denominator). When binary events give rise to proportions, it is a reasonable starting point to assume that errors will follow a binomial distribution (we revisit this initial assumption in Sect. 9.7.3). A traditional method to deal with binomially distributed errors is to transform the data prior to analysis by angular transformation (also known as arcsine square root transformation) but such transformation does not necessarily achieve normality, especially when many observations in the data set are of extreme proportions. Another problem is that this transformation discards information on the size of each data sample: an observation of two eggs parasitised out of a clutch of 10 stink-bug eggs will be given the same weight in the analysis as an observation of 20 eggs parasitised in a clutch of 100: although the two estimates of egg parasitism in this instance give the same value the latter is by definition a better estimate of egg parasitism rates in the underlying population because it is based on more information.

A better alternative is to use an analysis that initially assumes that the errors are binomially, rather than normally, distributed (Crawley, 1993; Wilson & Hardy, 2002; Briffa et al., 2013). Such

analyses are known as logistic regressions and there are logistic versions of the general linear models we have met so far. These work in essentially the same way as we have seen and, as with log-linear models (Sect. 9.7.1), the test of significance uses *G,* which is asymptotically χ^2-distributed under H_0.

The logistic regression can be described by:

$$y = \frac{1}{1 + \frac{1}{e^{(mx+c)}}} \tag{9.7}$$

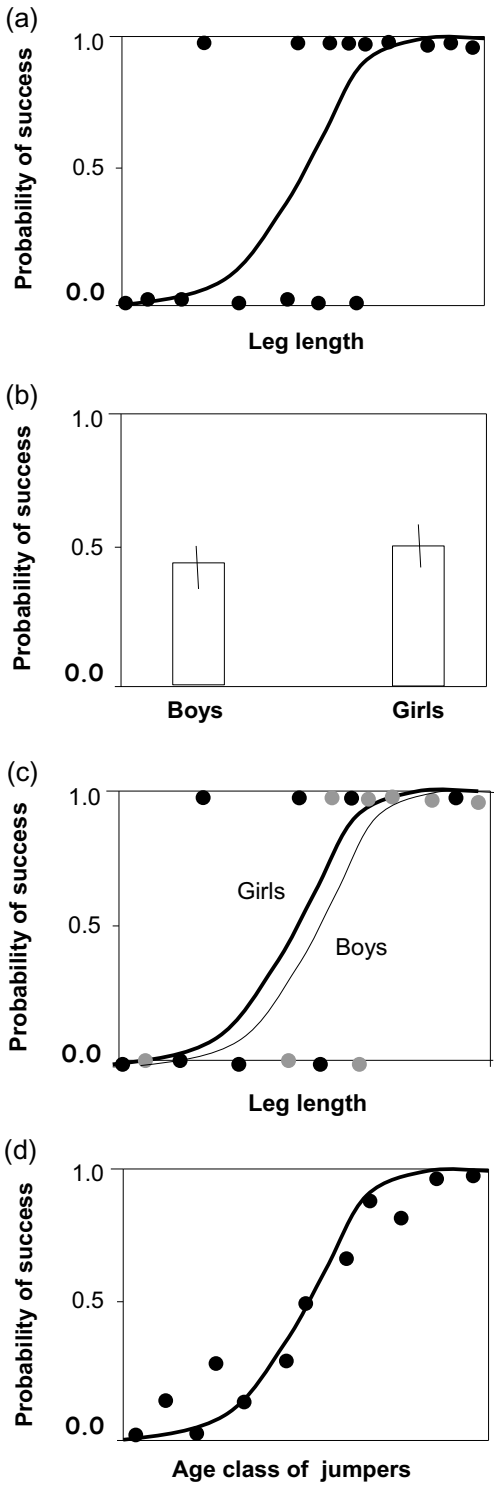
Again, this is called a logistic regression, because the link function is the logit function $\log(p/(1-p))$, where p is the probability that y=1.

At first glance this equation may seem rather complicated but is in fact just a modification of our original equation describing a straight regression line (Eq. 9.2). As with Eqs. 9.5 and 9.6 we have simply added a statement that the y values (the proportional response we are interested in understanding variation in) are predicted by a back-transformation of $mx + c$. In the logistic case this transformation is the antilogit, the '1/[1 + {1/(exp(x))}]' part, rather than the antilog which was used in Eq. 9.5. When the resulting regression line is plotted it can never fall below zero (just like with log-linear models) but can also not rise above 1. This makes sense because proportions must always take values between 0.0 and 1.0.

Binary data can be of two types: ungrouped or grouped. Ungrouped indicates that each output value, i.e., 0 or 1, comes from a separate single event (often called a 'trial'). Because there is just one event (denominator = 1) the resulting proportion must be either 1.0 (1/1) or 0.0 (0/1). Grouped binary data refers to cases where a number of events can happen simultaneously (denominator >1) and therefore output can take a proportion between 0 and 1. Ungrouped data is simply a special case of grouped data and is technically called a Bernoulli trial.

We, again, use a non-entomological and imaginary example to illustrate the approach for ungrouped binary data. Suppose we wanted to know whether the probability of individual school children being able to clear a 1.5 m high-jump is dependent on their leg length. For each child attempting the jump, we have measures of their leg length (a continuous variable) and whether or not they managed the jump, which is the binary response variable (0 = no, 1 = yes). Each child only had one attempt (Bernoulli trial) and there were 16 children. (We are using a relatively small imaginary sample size to ease illustration but note that the minimum recommended sample size for estimating an intercept in binary logistic regression is 96; Harrell, 2015.) We can carry out a logistic regression and obtain a graph like Fig. 9.22a. This shows that children with longer legs have a higher probability of successfully clearing the jump; the probability of success for the shortest-legged children is effectively zero whilst the longest-legged children almost always jump successfully, and there is a fairly steep transition in success as leg length increases beyond intermediate measures.

We could imagine dozens of other common scenarios to which we could apply such logistic analyses. For instance, whether the probability of individual parasitoids winning fights is dependent on their weight (dis)advantage compared to their opponents (Hardy & Field, 1998; Briffa et al., 2013; Hardy et al., 2013; Guerra-Grenier et al., 2020; Chap. 1), whether the probability of an emerging solitary parasitoid being male (or female) is associated with the quality (e.g., size) of its host (King, 1993; Wilson & Hardy, 2002; Karsai et al., 2006; Chap. 1), whether a male parasitoid manages to mate with females (Hardy et al., 2000; Mowles et al., 2013; Chap. 4), whether female parasitoids release volatile chemicals in response to contest intensity (Goubault et al., 2006), whether parasitoid species are pro-ovigenic (Ellers & Jervis, 2004) or whether the probability of a caught fish having an empty stomach is dependent on its geographic locality and/or its trophic level (Warton & Hui, 2011). These are all examples of binary responses that can be analysed by logistic regressions (because the explanatory variables are all continuous, such as leg length, contestant weight difference, host size).



◀ **Fig. 9.22** Illustrations of outcomes of logistic regression. **a** Simple logistic regression of ungrouped binary data. Note how individual outcomes are constrained to be either 0.0 or 1.0 but the estimated probability of success can take any value between and including these extremes. **b** Logistic one-way ANOVA-type: the height of each bar represents the overall (mean) success of boys or girls. **c** Logistic ANCOVA-type: combining (**a**) and (**b**): note that there is no (significant) interaction term as the two regression lines are shown parallel (they have the same shape and will never cross, even if used to predict success at more extreme values of leg length). **d** Simple logistic regression of grouped binary data. Here each data point represents the outcome of ten jumps (each by a different female of a given age) and thus the outcomes can take values intermediate to 0.0 or 1.0. We have shown a fitted regression line with the same slope as in other panels of this figure, but of course in a real example the lines would likely be different

We could similarly think of examples of binary responses to categorical explanatory variables which could be explored using the ANOVA-type approach in a logistic model, such as whether schoolgirls with a given leg length are better at clearing the high-jump than schoolboys, or whether fed or starved parasitoids tend to win contests for hosts (Snart et al., 2018). Figure 9.22b depicts the (imaginary) result that girls are a little (but significantly) more successful at the high-jump than are boys. We could, of course, explore simultaneously the effects of both leg length and sex on high-jump success using logistic 'ANCOVA-type' modelling: Fig. 9.22c depicts both the effect of leg length on high-jump success (the slope of the fitted regression lines) and the greater success of girls compared to boys of the equivalent leg length (the regression lines have different intercepts, which is most obvious at intermediate values of leg length).

Now let us imagine a similar set of grouped binary data. Suppose we took groups of 10 schoolgirls and female university students belonging to a range of age classes (i.e., 10 eight-year-olds, 10 nine-year-olds, etc., with twelve age classes overall, and thus a total sample of 120 females, aged 8–20 years) and asked each participant to attempt the 1.5 m high-jump once. As above we would obtain a binary response (0 or 1) for each individual but this time the individuals belong to age groups and for each age group, as

the denominator = 10 individuals per group, the possible proportions of successful jumpers are 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0. (If we had trialled only 4 jumpers per age group then our range of possible proportional responses would have been 0.0. 0.25, 0.50, 0.75 and 1.00). These data can be analysed using logistic regression in much the same way as the ungrouped data, but now each data point represents the combined performance of 10 individuals. Of course, trialling ten jumpers rather than, say, four, will likely give us a better estimate of jumping success, because it is based on more information. In logistic regressions, estimates from larger groups contribute more information than estimates from smaller groups, thus avoiding the disadvantage of equal weighting of information after angular transformation (see above).

Given that success in jumping a fixed-height bar is likely to increase with age (older jumpers will generally have longer legs, among possible other differences) we might expect the resulting graph to look something like Fig. 9.22d: most (but not quite all) young children (left side of the figure) cannot successfully clear the jump and success generally improves with age, such that university students (the age classes to the right of the figure) nearly always jump successfully. As with the ungrouped data, we could carry out more detailed explorations of factors that potentially influence success by gathering data on males as well as females, or on Chinese and Chilean jumpers etc., and thus run logistic versions of ANOVAs, ANCOVAs or multiple regressions, depending on the combination of continuous and discrete explanatory variables included.

To conclude this section we explicitly illustrate why standard regression analysis of binary proportional data is inappropriate. Figure 9.23a depicts a standard regression of grouped binary data, along with the assumed normal (Gaussian) distribution of errors at various points along the regression line. Notice that the graph has been drawn inside a box to emphasise the fact that the response variable is strictly bounded (proportions outside the range 0.0–1.0 are impossible). It can easily be seen that the standard regression

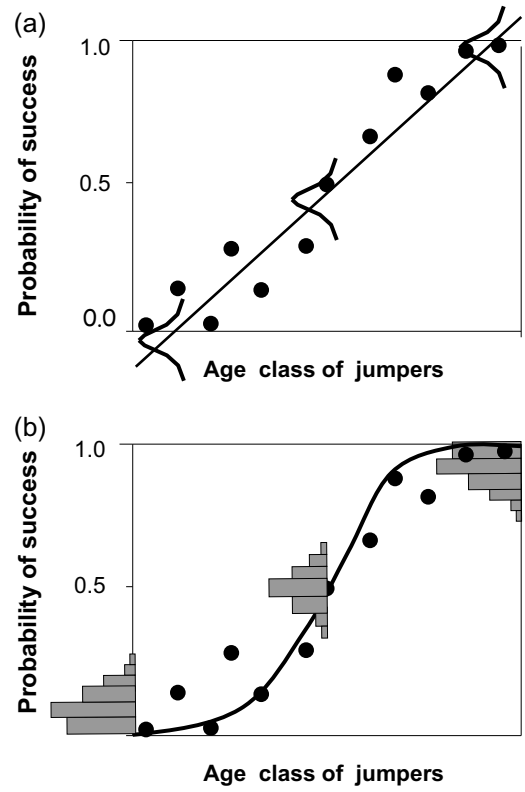


Fig. 9.23 Why the assumption of binomially distributed outcomes is appropriate for the analysis of proportional data. **a** A standard regression of grouped binary data (from Fig. 9.22d) assumes and predicts impossible proportions: i.e., the distribution of outcomes (bell-shaped histograms) and the fitted regression line (respectively) include proportions of less than 0.0 and greater than 1.0 (note that assuming a normal distribution is most reasonable for data on proportions close to 0.5). **b** These problems are resolved using a logistic regression as assumed distribution of outcomes, which changes shape according to probability (histograms) and the predicted proportions (regression line) remain within strict bounds

approach assumes that 'nonsense' proportions greater than 1 and less than 0 are possible. One tail of the normal distribution protrudes <0 when the proportion of successes is low and the other tail protrudes >1 when the proportion of successes is high. The assumption of normality is tolerable when proportions take intermediate values, around 0.5. Additionally, extrapolation of the relationship, which we might do if we wondered what would happen if we trialled girls of even older or even younger ages, also predicts

impossible proportions. These problems are solved by using a logistic regression that assumes binomially distributed errors (Fig. 9.23b). Because the binomial distribution lacks a left-hand tail at low mean values, impossibly low (<0) proportions are not assumed. Similarly, the lack of the right-hand tail at high proportions means that impossibly high (>1) proportions are not assumed when the mean is high. Extrapolation also brings no such problems since the regression line can never take values <0 or >1.

9.7.3 Logisitic and Log-Linear Model Checking

As with general linear models, it is common practice to perform model checking (Crawley, 1993) following logistic and log-linear analyses. This is because we have assumed that the errors are perfectly binomially or Poisson distributed (Sects. 9.7.1 and 9.7.2), but they may in fact not be. When analysing ungrouped binary data we are constrained to assume a perfect match to a binomial distribution, but for analyses of grouped binary data and of count data we can probe our initial assumptions and make subsequent adjustments.

Most statistical packages output the so-called 'heterogeneity factor' (HF), also called the 'scale parameter', 'scaling parameter' or 'dispersion parameter', estimated from the data to assess how well the data conform to a perfect Poisson or binomial distribution. If HF = 1 this indicates that the variance in the data is equal to what you would expect under the assumption of Poisson or binomial errors. HF < 1 indicates underdispersion, whereas HF > 1 indicates overdispersion. Although there are no fixed rules, HF > 1.5 is generally a cause for concern.

Overdispersion can indicate that data are somehow more clumped than random. In the case of count data it could be, for instance, that the fish species we have studied (Sect. 9.7.1) swim in shoals rather than as individuals (such that catches tend to being either zero or large numbers) and in the case of proportional data it could be that some groups of schoolgirls have received more high-jump training than others, making the binary responses of the girls within a group (Sect. 9.7.2) non-independent of each other. Overdispersion tends to be more common than underdispersion (Bourguignon et al., 2022), but underdispersion could arise in fish-count data if individuals maintained exclusive territories, leading to similar numbers of fish caught by each standard sweep of the net (Sect. 9.7.1). For proportional data we could imagine that the 10 schoolgirls and university students in each age group were chosen non-randomly to represent the full spectrum of abilities within each age group by an egalitarian teacher, leading to lower than binomial variation in abilities across groups. Among natural enemies, evolutionary natural selection favours low variation (underdispersion) in the sexual composition of offspring groups for parasitoids with local mating systems, while developmental mortality of some offspring within those groups can lead to overdispersion (Krackow et al., 2002; Kapranas et al., 2011; Wilkinson et al., 2016).

We need to be aware of these issues because overdispersion can lead to Type I errors whereas underdispersion may cause Type II errors (Table 9.1). Both over- and under- dispersion can be dealt with by replacing the assumed value of HF (this will be 1 by default in most statistical software) with the actual value (estimated by the ratio of the residual deviance to the residual df), and re-fitting the model based on the assumption of quasi-Poisson or quasi-binomially (i.e., a Poisson or a binomial distribution with a dispersion parameter added) distributed errors, then testing significance using *F*-tests) (e.g., Hardy & Cook, 1995; Cusumano et al., 2022; Liu et al., 2023). Note that this process does not change the estimates of the slope and the intercept but their standard errors are increased (for overdispersion) or decreased (for underdispersion), which in turn affects the calculated *P*-value. Adjusting for overdispersion thus has the effect of making it more difficult to obtain a significant result from the data (and thus reduces Type I error rate) while adjusting for underdispersion makes significance more likely (fewer Type II errors). We can make the analytical process more straightforward by simply using empirically estimated distributions

straight away, rather than first assuming perfect Poisson or binomial distributions and then adjusting them (e.g., Kapranas et al., 2011; Holmes et al., 2023). This is easily done in statistical packages (e.g., GenStat, GLIM, R) and we recommend doing this before attempting to normalise variables by transformation or resorting to non-parametric analyses (see Bourguignon et al., 2022, for further possibilities). Note, however, that we cannot adjust for under- or overdispersion when analysing ungrouped binary data (Crawley, 1993; Wilson & Hardy, 2002): as mentioned above, we just have to assume that the data are truly binomially distributed.

On a somewhat philosophical note, we regard estimating the scale parameter empirically as the next level of analytical sophistication above that of assuming non-normality. This is because it is another step towards making the statistical test fit the way that the data were originally collected rather than making the data fit the assumptions of a test.

9.8 Beyond This Chapter

We have now covered a range of parametric techniques, for which the collective terminology is summarised in Fig. 9.24. You may have skipped ahead to look at this when reading Sect. 9.3: whether or not you did, it will probably repay some contemplation at this stage. We have

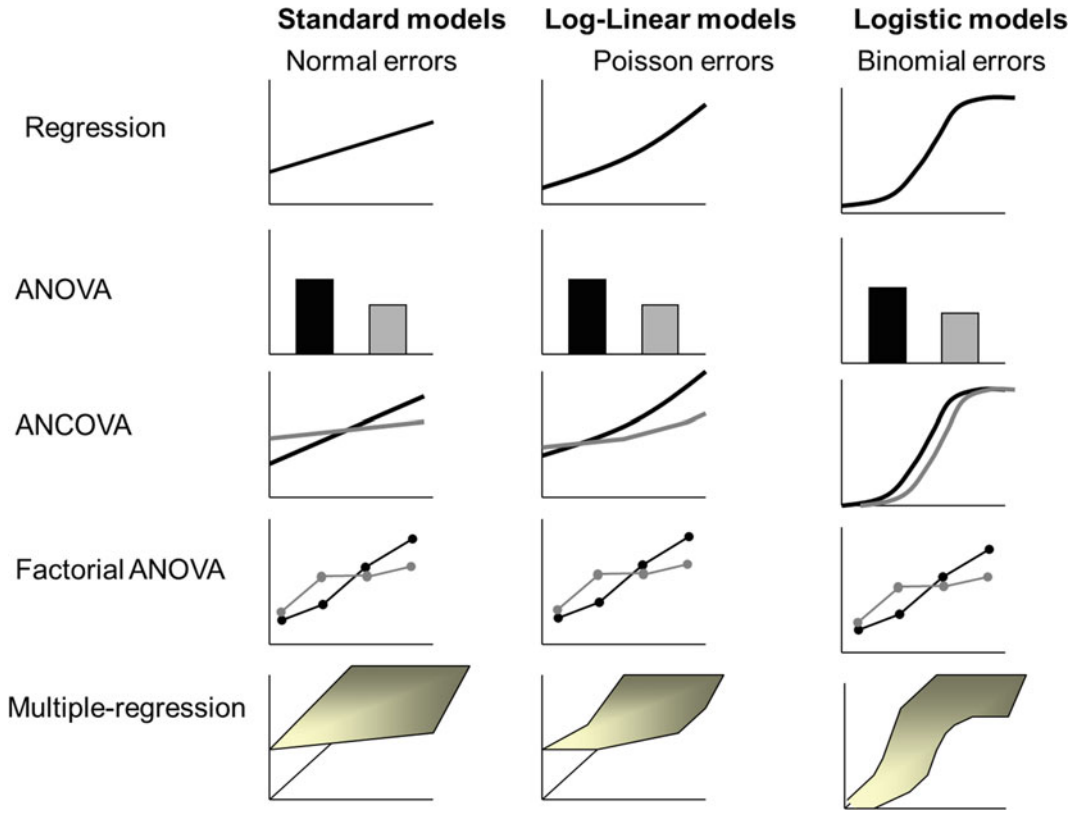


Fig. 9.24 Schematic overview of the generalised linear model (GLM) family of techniques using icons to denote the typical form of graphs resulting from these analyses. The sub-family of 'standard' techniques assuming Gaussian (normally distributed) errors, general linear models, forms the left-hand column with the other columns representing the sister techniques which adopt different assumptions about the distribution of errors for situations in which these are likely to be non-Gaussian. Note that there are further members of the GLM family that are not included in this representation

found this visualisation extremely useful, and we have not seen it explicitly given elsewhere.

An understanding of the generalised linear model (GLM) family provides a platform for expanding one's range of statistical abilities to include yet further techniques, and thus the end of this chapter can also be the beginning of much more. These are at least three interrelated reasons for this. Firstly, many of the concepts we have met during the chapter are generic rather than particular to GLMs and this pre-adapts us to learn further statistical methodologies (many of which are to be found in, for example, Harvey & Pagel, 1991, Sokal & Rohlf, 1995, Quinn & Keough, 2002, and Roff, 2006) or understand, at least in essence, analyses presented in papers and reports we need to read, write and evaluate.

Secondly, and more directly, there are many additional techniques that can be used within the GLM framework but that we have not covered in any detail here: examples include the use of polynomial terms and splines to model non-linearities, the range of variations of the ANOVA approach, for instance those that include blocking (e.g., Quinn & Keough, 2002), analysis of data with gamma-distributed errors (e.g., parasitoid development times: Holmes et al., 2023 parasitoid patch residence times: Rohlfs & Hoffmeister, 2004; parasitoid time to mating and mating duration:Charrat et al., 2023), negative binomial errors (e.g., egg parasitism rates: Cornelius et al., 2021) or cohort survival analysis (Crawley, 1993; Zhang, 2016; Chap. 2). We have found the latter to be particularly useful in studies of parasitoid reproductive performance (recent examples include, Amante et al., 2017, Lupi et al., 2017, Snart et al., 2018, Jucker et al., 2020, Abdi et al., 2021, and Malabusini et al., 2022).

Thirdly, some new techniques we may meet are further generalisations of GLMs themselves. For instance, the GLMs we have covered allow us to test the effect of one or more explanatory variables on a single response variable. Sometimes, however, we may want to test the effects of one or more explanatory variables on multiple response variables as well as interactions between response variables. Techniques that do this are called multivariate analyses, such as multivariate analysis of variance (MANOVA) (Quinn & Keough, 2002). Many statistical packages allow multivariate analyses and the results can be interpreted in much the same way as those for GLMs. Examples of natural-enemy studies that have employed MANOVAs include Khidr et al. (2013), Morrison et al. (2018), Bui et al. (2020), Aspin et al. (2021), and Velasco-Hernandez et al. (2021).

Another technique that is being increasingly used, and which builds on GLMs, is generalised linear mixed modelling (GLMM, e.g., Bolker et al., 2009): this can explore both fixed effects and also random effects on a single response variable. Fixed effects are the type of explanatory variables, and their interactions, that we have covered in this chapter so far. Random effects are variables (e.g., the identity of each organism or population contributing to a set of data) that are not experimentally replicable or fully controllable, due to inter-individual variation or the differing environments in which populations are found (e.g., Krackow & Tkadlec, 2001; Bolker et al., 2009). They can also consider the fact that we sometimes have to handle a set of different levels of a factor that are just a random choice of an infinity of possible levels. For example, we may wish to consider the 'species effects' on a binary outcome or on an integer response, but we will likely only have a random sample of species drawn from all possible species that could be studied.

For many scientists the most familiar example of a random effect will be blocking, as used to reduce nuisance environmental variation by traditional agricultural experimental designs that employ variants of ANOVA. GLMMs provide an alternative method for dealing with overdispersion due to between-group variation (Sect. 9.7.3, Krackow & Tkadlec, 2001; Wilson & Hardy, 2002; Briffa et al., 2013) as well as modelling non-independent observations. We have used GLMMs in several recent studies of natural-enemy biology (e.g., Abdi et al., 2020; Guo et al., 2022), as have others (e.g., Vanbergen et al., 2007; Do Thi Khanh et al., 2012; Villacañas de Castro & Thiel, 2017; Liu & Hao, 2019; Guerra-Grenier et al., 2020; Cornelius

et al., 2021; Charrat et al., 2023; Liu et al., 2023) and, philosophically, regard them as the next level of analytical sophistication above GLMs. This is because they neither ignore random effects nor treat them as if they were fixed effects.

GLMMS are conditional models that lead to inferences about how individuals respond to a given set of circumstances, taking their non-independence (random effects) into account. An alternative is to use the generalised estimating equation (GEE) (Liang & Zeger, 1986; Pekár & Brabec, 2018). GEEs are marginal models that take into account non-independent data and lead to inferences on how population averages respond, with no emphasis on the random effects. Natural-enemy studies that have used the GEE approach include Fauvergue et al. (2007), Le Lann et al. (2011), Alvarez-Baca et al. (2020), and Volter et al. (2022).

9.8.1 Hypothesis Testing Revisited

We began this chapter with the concept of hypothesis testing, the traditional and still most commonly used statistical approach (Sect. 9.2.2). Let us finish by noting that there is a view that, when considering a body of evidence in the published literature, less attention should be given to the results of null-hypothesis significance testing, which leads to noting that study A concluded X and study B concluded Y (e.g., 'vote counting'), especially when sample sizes within each study are small. Rather, the focus should be more on what the general trend has become as studies accumulate, which can be achieved by meta-analysis. Meta-analysis is a systematic review of the literature which incorporates the effect sizes and confidence intervals of individual studies to yield an overall summary effect (e.g., Gates, 2002; Stewart, 2010; Yang et al., 2023) and a measure of heterogeneity of effects. The effect size represents the strength of the relationship (or 'the degree to which the phenomenon is present in the population'; Cohen, 1988; see also Nakagawa & Cuthill, 2007) and can be simply thought of as the 'steepness of the slope' (regression) or the 'extent of the difference' (ANOVA). In meta-analysis, the *P*-value for the summary effect is substantially more compelling than that of any single study (Borenstein et al., 2009). Meta-analysis is becoming more common and is widely seen as more informative than traditional narrative or vote-counting reviews. Traditional null-hypothesis testing does not include reporting of either the effect size or the precision of the estimate of effect size, and, as such, both are now encouraged when reporting results within individual studies in order to facilitate future meta-analyses (Nakagawa & Cuthill, 2007; Halsey, 2019; Charrat et al., 2023) that will provide overview evaluations of important scientific questions.

9.9 Conclusions

Scientific research, including studies of insects as natural enemies, is fundamentally about testing hypotheses, and, to do that, we need statistical analyses. Inferential statistics allow us to assess sample data sets for significant differences or associations among observed measures and the principle of parsimony often plays a central role. Parametric approaches are powerful and flexible and are very commonly used. General linear models include regression, ANOVA, ANCOVA, factorial ANOVA and multiple regression, all of which assume a normal error distribution. General linear models are a sub-family within the wider family of statistical methods known as generalised linear models (GLMs, sometimes called GLZs) which all work, at least conceptually, in the same way. GLMs include log-linear and logistic alternatives for each of the general linear models. These can be appropriate when errors are non-normally distributed. An understanding of the GLM family provides a solid platform for expanding one's statistical repertoire yet further to incorporate techniques such as GLMMs and many others.

Acknowledgements Statistics is a continually developing field: we managed to reach some sort of agreement for most of the aspects on which we initially seemed to differ with those who helped us develop this chapter: any remaining inaccuracies or misunderstandings may well be

ours. We recognise that, in parts, the terminology we use might aggravate some practising statisticians: our aim has been to provide a non-technical introduction. If you happen to find it overly simplistic, bear in mind that colleagues and students who had struggled with statistics have greatly appreciated having access to early drafts: this is for them and for others like them.We are very grateful for the constructively critical comments, on various drafts, of Eric Wajnberg, Richard Wilkinson and Nick Galway. We are also grateful to several further colleagues for numerous interactions over statistical issues: Peter Alderson, Jim Craigon, Martin Gammell, Charlie Hodgman, Chungui Lu and Debbie Sparkes spring particularly to mind as regards the history behind this chapter, which was begun at the Sutton Bonington Campus of the University of Nottingham, UK. The chapter has even deeper roots, as it follows the approach of *GLIM for Ecologists* by M. J. Crawley (1993). In his book M. J. Crawley thanked his colleague's neighbour for introducing his colleague to GLIM and, in turn, his colleague for passing on the information. ICWH now thanks M. J. Crawley for his role in the further dissemination of the GLM approach and DRS in turn thanks ICWH. We hope that you will, in the course of time, thank DRS. As it says in the Preface, pass it along.

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Genus and Species Index

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Subject Index

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