Naturwissenschaften (2001) 88:147–158
DOI 10.1007/s001140100222

REVIEW ARTICLE

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On the evolutionary ecology of host–parasite interactions: addressing the question with regard to bumblebees and their parasites

Published online: 8 May 2001

Abstract Over the last decade, there has been a major shift in the study of adaptive patterns and processes towards including the role of host–parasite interactions, informed by concepts from evolutionary ecology. As a consequence, a number of major questions have emerged. For example, how genetics affects host–parasite interactions, whether parasitism selects for offspring diversification, whether parasite virulence is an adaptive trait, and what constrains the use of the host's immune defences. Using bumblebees, *Bombus* spp, and their parasites as a model system, answers to some of these questions have been found, while at the same time the complexity of the interaction has led expectations away from simple theoretical models. In addition, the results have also led to the unexpected discovery of novel phenomena concerning, for instance, female mating strategies.

The questions

When the Spanish conquered Central and South America in the 16th century, they not only brought cannons and horses with them, but also a large number of invisible but very efficient weapons – the germs of the Old World, such as smallpox, which crossed the Atlantic sometime in 1518 or 1519. Smallpox is a terrifying disease and was devastating for the indigenous people of the New World who had never been exposed to this pathogen before. Thus, parasites probably added more to the success of Cortez and Pizarro than firearms ever did (Crosby 1986). As this historical note vividly demonstrates, there can be little doubt that parasites play a subtle but critically important role for the host.

Indeed, a major conceptual shift in the study of adaptive phenomena in behaviour, ecology and evolutionary biology over the last decade has been to consider the role of host–parasite interactions. Several major questions, at various levels of analysis, have thereby emerged. For example, to what extent can parasites have a major impact on the structure of the communities of their hosts (Dobson and Hudson 1986; Price et al. 1986, 1988; Minchella and Scott 1991)? If co-existing host species showed different susceptibilities or parasite loads, this fact could give one host species a decisive competitive edge over another (Feener 1981). Similarly, parasites could affect patterns of reproduction and life history variation in many ways (Minchella 1985; Godfray and Hassell 1987).

On a more immediate level, any study of host–parasite interactions must also address the potential harmful effects of the parasite on its host. On one hand, this simple question is not always easy to answer, because the negative effects are not always clear-cut and immediately visible. In addition, parasite effects often depend on host condition and may only be expressed when the host is in a poor state due to other reasons. On the other hand, this question has important ramifications when it comes to studying the functional, evolutionary reasons of parasite virulence: why is one parasite comparatively benign while another parasite species is devastating for the host. The hypothesis that virulence is an adaptive trait which evolves to maximize the parasite's reproductive rate, in particular, to maximize transmission to new hosts, has been very instrumental in developing the conceptual framework. In fact, over the last decade, theory has made substantial progress in this area (Bull 1994; Ebert 1998a), but the empirical evidence has not been conclusive. Clearly, if we had a good understanding of what drives the evolution of parasite virulence, a wealth of useful applications could emerge from this, for example, in agriculture and medicine.

Host–parasite interactions appear to have some characteristics that set them apart from many other ecological interactions between species. Among those, the strong effect of genes is particularly relevant. As Haldane (1949, p.70) noted, rather figuratively, "...it is much easier for a mouse to get a set of genes which enable it to

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resist *Bacillus typhimurium* than a set which enable it to resist cats". Although the various defences against a predator, such as a good sense of smell, acute hearing and anti-predator behaviours are all based on genes, the interaction with parasites, especially micro-parasites such as viruses, bacteria, fungi and protozoa, is in this sense presumably more direct. Indeed, virtually all the studies that have addressed this question have found genotypic variation in the expression of parasite infectivity and host susceptibility (e.g. Wakelin and Apanius 1997). This pattern has important consequences that Haldane (1949) already envisaged: if the interaction between host and parasite primarily depends on their genotypes, then parasites may ultimately be responsible for the maintenance of the large amounts of genotypic variation that we observe in natural populations (and which otherwise would be quickly eroded by natural selection). This question has become a major focus for research. Can parasites maintain genetic diversity in their host and, conversely, does genetic diversification of offspring provide an advantage in a parasite-ridden world? This issue is connected at a deep level to the question of why sexual reproduction evolved and what maintains it against the clonal alternatives (Bell 1982; Lively 1996). It has also been suggested that the precise nature of the genotypic interaction, for example, as a gene-for-gene or a matching allele model, affects the outcome of these processes, which shows the importance of investigating the details of this interaction in a given study system.

These ideas concentrate on the ways in which hosts may mitigate the effects of parasitism and have been captured in the Red Queen scenario (Bell 1982; Hamilton et al. 1990; Lively et al. 1990). However, hosts have an additional defence line, the immune system, which is able to identify an invader and to combat the infection. Of all bodily functions, the immune system, especially that of vertebrates, is certainly among the most complex and sophisticated. In the context of the increasing interest in the evolutionary ecology of host–parasite interactions, new approaches to the study of immune defences have emerged. In particular, a priori reasons suggest that the evolution of the immune system compromises other components of an organism's fitness (Sutter et al. 1968; Kraaijeveld and Godfray 1997). With the immune system in place, its use should be costly too, similar to the use of muscles to forage or the use of nerve cells to process information. But just how costly could it be and are these costs expressed in any relevant context? And, if the costs exist, do organisms use the immune system in an efficient way, which implies that it should not always be deployed at the maximum rate (e.g. Jokela et al. 2000)?

The prospects generated by these questions for a deeper understanding of organic diversity are exciting. The research programme itself, however, is not without its difficulties. It requires inter-disciplinary work in evolution, ecology, behaviour, genetics, parasitology and, last but not least, theoretical modelling. In addition, not many actual systems have proved suitable for carrying out laboratory and field studies at the same time and for allowing experimental manipulation in a satisfactory manner. Amazingly, bumblebees and their parasites represent one such system. They have been studied by us since the mid-1980s. Although many open questions still remain, these studies have provided deep insights into how hosts and parasites interact in a real system and what consequences this interaction may have for both parties. In addition, several unexpected lines of research have been generated. For example, new insights into the mating behaviour of females and selfish strategies of males have been gained. Furthermore, as bumblebees are social animals, contributions which clarify the significance of parasites for the evolution and maintenance of sociality have also been made.

The natural history of the host

Bumblebees are large-sized pollinators of temperate areas and have colonized all parts of the World except sub-Saharan Africa and Oceania (where, however, they have been introduced by man). All of the several hundred species that have been described share a similar life history and ecology (e.g. Alford 1975) (Fig. 1). The inseminated queens diapause, typically in the soil, under leaf litter or above ground, in hollows in trees or buildings, in order to survive the unfavourable season. The queen emerges from her diapause at the start of the season, that is, in spring in temperate areas. She will forage for nectar and pollen, develop her ovaries, locate a suitable nesting site and start a colony by laying her first batch of eggs into the pollen lump she has prepared. A few weeks later, the first generation of workers, her daughters, will hatch and start to take over the work of the newly founded colony.

As the season progresses, more and more workers will be added to the colony, the numbers being rather

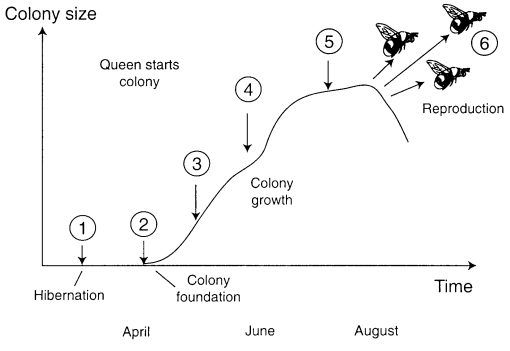

Fig. 1 Life cycle of bumblebees (*Bombus* spp.). The trypanosome parasite, *Crithidia bombi*, is thought to have an effect on its host as follows: (*1*) Hibernation survival not affected (P. Schmid-Hempel, unpublished data); (*2*) Founding success reduced (M. Brown et al., unpublished data); (*3*) Early colony growth reduced (Shykoff and Schmid-Hempel 1991c); (*4*) Increased worker mortality under adverse environmental conditions (Brown et al. 2000); (*5*) Delayed reproduction (Shykoff and Schmid-Hempel 1991c); (*6*) Infections may affect the build-up of the queen's fat body for hibernation

variable among different species. *Bombus terrestris* L., the main species studied here, will typically live in colonies of 100–200 workers at any one time although colonies can have as few as 10 and as many as 1,000 members, depending on year and weather conditions. Towards the latter part of the season, the colony starts to reproduce, that is, sexually competent daughter queens and males (in social bees, males are often called drones) are produced instead of more workers. What exactly determines the point in time when sexuals are raised and whether this transition is sudden or gradual is a matter of ongoing debate (e.g. Duchateau and Velthuis 1988; Müller et al. 1992). Whatever the exact pattern, the sexuals will leave the nest, mate, and finally the inseminated daughter queen enters diapause while all other colony members perish as autumn approaches. The entire life span of the colony, from the emergence of the first workers to reproduction, is around 2–4 months. This makes it possible to cover the life cycle and measure fitness of colonies (and thus the fitness of the founding pair) in a reasonable time span.

The parasites

Bumblebees are hosts to a large number of parasites (Table 1). For most of these, very little or next to nothing is known about their effects, epidemiology or evolutionary ecology. In addition to the parasites listed in Table 1, bumblebees are also host to a large number of mites. For example, a single survey in an area near Zurich identified ten species of mesostigmatic mite alone on a total of 141 spring queens inspected (Schwarz et al. 1996). The most common species were *Parasitellus fucorum* and *Hypoaspis bombicolens*, while mite prevalence varied among host species. Most mites are probably not truly parasitic but rather live on debris in the nest or consume some of the nest provisions. At least one species, the tracheal mite, *Locustacarus buchneri*, can be extremely damaging for the affected individual and may put the entire colony in peril. A few parasites have been studied in more detail. For example, two species of conopid fly, *Sicus ferrugineus* and *Physocephala rufipes*, attack various bumblebee species and can be extremely prevalent,

Table 1 Parasites reported for bumblebees (after Schmid-Hempel 1998)

Group	Parasite	Remarks
Virus	Acute Bee Paralysis virus Entomopox virus	Uncertain status in nature
Bacteria	*Spiroplasma* *Aerobacter cloaca* and other unidentified bacteria	In haemolymph
Fungi	*Acrostalagmus* *Beauveria bassiana*, *Candida* *Hirsutella*, *Metarhizium*, *Paecilomyces*	Possibly shortens hibernation
Protozoa	*Apicystis bombi* *Crithidia bombi* *Nosema bombi* *Neogregarina* sp.	Can completely destroy the fat body A study object Can kill entire colonies but highly variable in expression. A creeping disease
Nematodes	*Sphaerularia bombi*	Infects hibernating queens and castrates them. Known from almost all species
Hymenopoteran parasitoids	*Syntretus splendidus* *Melittobia acasta*; *M. chalybii* *Monodontomerus montivagus* *Pediobius williamsoni*	Probably attacks spring queens exclusively
Dipteran parasitoids	*Apocephalus borealis* *Boettcharia litorosa* *Helicobia morionella* *Brachioma devia; B. sarcophagina; B.setosa* *Conops algirus; C. argentifacies; C. elegans; C. flavipes; C. quadrifasciatus; C. vesicularis* *Melaloncha* sp. *Physocephala brugessi; P. dimidiatipennis; P. dorsalis; P.nigra; P. obscura; P. rufipes; P. sagittaria; P. tibialis; P. vittata* *Senotainia tricuspis* *Sicus ferrugineus* *Zodion* sp.	Feeds on thoracic muscles Can be extremely destructive Investigated, particularly in *B. terrestris* Pupa can be hyper-parasitized by pteromalid wasps Investigated, particularly in *B. terrestris*
Lepidoptera	*Ephestia kühniella*	Feeds on provisions
Acari (mites)	A large number of species	Unclear status as parasites

Table 2 Some findings on the interaction of bumblebees with parasitic flies (Conopidae, Diptera)

Finding	Source
Parasitization by conopid flies is common and affects various host species	(Schmid-Hempel et al. 1990; Shykoff and Schmid-Hempel 1991a)
Different conopid fly species show host choice with respect to species and sex (lower preference for males). This seems to correspond to their respective developmental success	(Schmid-Hempel et al. 1990; Schmid-Hempel and Schmid-Hempel 1996)
Conopid flies prefer large hosts	(Müller et al. 1996; Schmid-Hempel and Schmid-Hempel 1996)
Host is killed within 10–12 days after oviposition	(Schmid-Hempel and Schmid-Hempel 1996)
Conopid-induced mortality may take effect mainly early in the season, since colony size at the time is a good predictor of reproductive success	(Müller and Schmid-Hempel 1992a; Müller and Schmid-Hempel 1993a)
Parasitized hosts prefer to visit different plant species than their non-parasitized counterparts	(Schmid-Hempel and Schmid-Hempel 1990; Stauffer and Schmid-Hempel 1998)
Parasitized hosts change behaviour and stay outside the nest in the cold at night. This serves to prolong host lifespan	(Müller and Schmid-Hempel 1993b; Müller and Schmid-Hempel 1992b)
Parasitization causes dying hosts to dig themselves into the ground. This serves to increase the parasite's chances to survive the winter	(Müller 1994)
The timing of reproduction in host populations correlates with prevalence of parasitization by conopid flies	(Schmid-Hempel and Durrer 1991)

with more than half of all workers in the field infected. Several years of study have uncovered many details of this interaction (Table 2).

Among the protozoan diseases of bumblebees, *Nosema bombi* is reasonably common (Shykoff and Schmid-Hempel 1991a; Macfarlane et al. 1995). This could be described as a "creeping" disease, because even when *Nosema* is present in the overwintered queen, the infection will often not become visible until much later in the seasonal cycle when workers start to die of the parasite. *Nosema* shows strong interactions with the host, *B. terrestris*, most probably based on host–parasite genotype–genotype interactions, with large amounts of variation among colonies (Schmid-Hempel and Loosli 1998). In addition, *N. bombi* can be cross-infected to other species (from *B. terrestris* to *Bombus lapidarius* and *Bombus hypnorum*), although it is less infective in the foreign species. *Nosema bombi* is clearly different from *Nosema apis*, which infects the honeybee (McIvor and Malone 1995). Worker and male larvae and adult workers can all become infected by *N. bombi* spores, but infections may have quite unexpected results. For example, in a study extending over their entire life cycle, colonies that naturally acquired *Nosema* infections produced more (rather than less) daughter queens and males than uninfected colonies did (Imhoof and Schmid-Hempel 1998a). Although there is little doubt that *Nosema* can be extremely devastating for individuals and entire colonies (personal observation), there seems to be no simple pattern.

Addressing the questions with a micro-parasite

Effects and transmission of *Crithidia bombi*

The protozoan *Crithidia bombi* (Lipa and Triggiani 1980) is a flagellated trypanosome that inhabits the gut of bumblebees. The cells attach to the gut wall where they multiply. A few days after infection, the cells are passed out in the host's faeces and can infect others (Schmid-Hempel and Schmid-Hempel 1993). The spread of the infection within the colony is easily accomplished when the next host comes into contact with infective cells on the brood comb or other nest materials. Because bumblebees have no trophallaxis (the passing on of food via regurgitation), infectious pathogens like *Crithidia* cannot be transmitted directly but are picked up from surfaces in the nest.

In contrast to transmission within the nest, the spread of the infection between colonies is more difficult. It is possible that infected workers wander off to other nests which they erroneously enter and become accepted (Sakofski and Koeniger 1988). The more important route for transmission, however, appears to be via flowers. Experimental studies have demonstrated that infections are passed on by visitors to flowers, such that a healthy bee can pick up the infection previously deposited by an infected forager (Durrer and Schmid-Hempel 1994). Although not perfect, the transmission happens in 20–40% of cases. In addition, the likelihood of becoming infected appears to depend on the architecture of the inflorescence. Transmission on simple inflorescences, for example, when all flowers are arranged in a simple row, is more likely than on more complex inflorescences, such as when flowers spiral up the stalk (Durrer and Schmid-Hempel 1994).

In the field, the prevalence of *C. bombi* among workers is quite high (the majority are typically infected) but varies among host species, localities, and time of year. Almost all colonies in the field are infected but not every worker within a colony is. As the season progresses, the chances that a colony has become infected increase dramatically. For example, in an experiment, previously uninfected colonies were exposed to the field situation at different times of the year. The later the field placement took place, the shorter was the time until the first sign of infection in the colony was found subsequently. Eventually, at the beginning of July, a healthy colony would become infected within a few days of being exposed to field conditions (Imhoof and Schmid-Hempel 1998a). This dramatic increase in the force of infection is likely to be caused by an increasing number of infected colonies in the population, combined with an increasing number of workers in each of these colonies that are able to transmit the disease during visits to flowers (Durrer and Schmid-Hempel 1994).

Crithidia bombi is probably a typical parasite, since its effects are not always dramatic. For example, experimentally infected workers normally show no dramatic increase in mortality. Only when workers are stressed, for example, by restricted access to food, will effects occur. With starvation, the mortality rate can be increased by more than 50% (Brown et al. 2000). This clearly is a substantial effect. Furthermore, stressful conditions, such as food shortage, are likely to be common in young colonies in spring. During this time, variable and adverse weather and the small number of workers makes it difficult for the colony to collect enough resources. We should therefore expect *C. bombi* to take a heavy toll early in the season. At least in the laboratory, (naturally) infected colonies grow more slowly and their worker population size is smaller for the first, and crucial, 25 days of the colony cycle. The demography of infected colonies is also different, as the average age of the workers is lower (Shykoff and Schmid-Hempel 1991c). However, under (benign) laboratory conditions, hardly any effect of naturally acquired infections on the reproductive success of the entire colony can be found. On the other hand, the infection affects the social fabric of the colony. Typically, some workers escape the queen's control and manage to lay their own eggs. Particularly towards the end of the colony cycle, this reproductive conflict (that is, the conflict over who can lay own eggs) between the queen and her workers becomes evident (Duchateau and Velthuis 1988). It turns out that workers in infected colonies do not manage to lay own eggs as early as workers in uninfected colonies do (on average, 5 days earlier). Because infection delays the conflict between the queen and her workers, in some sense infected colonies can be considered to be more “social”, that is, more co-operative. In addition, in the infected colonies the first daughter queens and males appear a few days later than they do in the healthy colonies (Shykoff and Schmid-Hempel 1991c). This is a small difference at first sight but a difference that may have large effects on queen survival during hibernation. For as yet unknown reasons, the chance to survive to the next year decreases rapidly for queens that are born later in the season (C. Gerloff and P. Schmid-Hempel, unpublished data).

The difficulty in demonstrating clear effects of an experimental infection in the laboratory therefore does not imply that *C. bombi* is an insignificant parasite in the field. On the contrary, there are several findings, discussed below, that suggest otherwise. Among other things, it seems that the chances for a spring queen to found her colony at the appropriate time are significantly reduced if she is infected by *C. bombi*. Unfortunately, the exact reasons are not yet known (M. Brown et al., unpublished data). This parasite thus affects the success of the queen in drastic ways even before a colony life cycle starts (Fig. 1).

Genotypic interactions

Infection experiments with *C. bombi* and its host, *B. terrestris*, have clearly shown that a large amount of variation is present in this interaction. When *C. bombi* is extracted from different colonies of origin and these inocula are fed to host workers of a particular target colony in controlled ways, there are large differences in infection rate. Some sources will infect very easily, whereas other sources will produce little or no infection. Therefore, infection depends on where the parasite comes from (and into which colony it goes). The study of this interaction benefits from two major technical achievements. Firstly, *C. bombi* can now be cloned from single cells (Wu and Schmid-Hempel 1997) which makes it possible to use inocula of single clones. Secondly, several primers for micro-satellite loci have been discovered (P. Schmid-Hempel and C, Reber, unpublished data), which makes it possible to analyse the genetic structure not only of the host but also of the parasite population.

The genetic architecture of individual trypanosomes, and of other protozoan parasites, is largely unknown but where investigated appears to be complex. In particular, there is no general agreement on what kind of population structure – sexual or clonal – these parasites exhibit (Tibayrenc 1995; Gibson and Stevens 1999). In the case of *C. bombi*, enzyme electrophoresis (Wu 1994) as well as micro-satellite analysis (P. Schmid-Hempel and C. Reber, unpublished data) suggest that it is a diploid trypanosome. However, it is not yet known with certainty whether genetic exchange (i.e. a kind of sexual reproduction) exists in *C. bombi* or whether it is a purely clonal species. According to results obtained so far, *C. bombi* appears to be primarily clonal. No striking evidence for sex has yet been discovered. Therefore, we can adopt the working hypothesis that a population of *C. bombi* consists of different “strains” which, if anything, show low levels of genetic exchange between them.

Experimental infections under controlled conditions with different clones or with different sources of *C. bombi* in workers from different colonies yielded clear

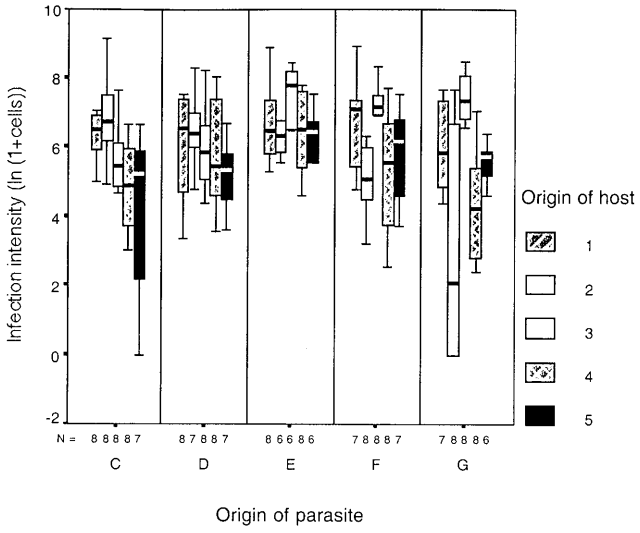

Fig. 2 Infection intensities (cells/µl of faeces, ln-transformed) of *Crithidia bombi* in experimentally infected workers of *Bombus terrestris*. In each panel, the graph shows the median (*horizontal bars*), the ± 25% quartiles (*boxes*), and lowest/highest values (*vertical lines*), respectively, of the observed intensity (averaged over *n* workers as indicated at the bottom). For the infections, the same five parasite lines were used ("*origin of parasite*": five strains, *C...G*, derived from independent sources were extracted and cloned for this purpose). These five strains were infected, one per host, into workers coming from five different colonies ("*origin of host*": colonies *1...5*, indicated by *different shadings* in the graph), such that all possible combinations of host line and parasite line were tested. The workers were infected with an inoculum of a total of 50,000 cells applied in 10 µl of sugar solution. The resulting infection intensity was then assessed from faeces samples 5 days later. A total of 186 workers were tested. In this experiment, infection intensity is not affected by host origin (ANOVA; random factor colony: $F(4,16)=2.175$, $P=0.118$), parasite origin (random factor strain: $F(4,16)=1.409$, $P=0.276$), but significantly so by their interaction (interaction term: $F(16,161)=2.178$, $P=0.008$) (from P. Schmid-Hempel and R. Loosli, unpublished data)

results. In all studies, a significant interaction between origin of host and parasite for the resulting level of infection was found (see example in Fig. 2). Using microsatellite markers for the parasite, the existence of considerable host–parasite genotype–genotype interactions can also be shown directly. When an experimental "cocktail", consisting of infective cells from different sources of *C. bombi*, was first infected into and then transmitted from workers of a donor colony to workers of a target colony, the identities of both source and target colonies, were crucial for the eventual infection in the targeted workers. Genetic analysis showed that the alleles contained in the original cocktail arranged themselves, such that the number and identity of alleles present in the targeted workers depended on the actual transmission from-to pathways (Schmid-Hempel and Schmid-Hempel 1993). This suggests that, in the field, the different parasite strains may infect different host family lines and that there is substantial genotypic variation underlying the observable infection patterns in the wild which, combined with the selective effects, is likely to generate processes of local co-evolution.

Theoretical studies have considered the co-evolutionary dynamics of such local interactions between hosts and parasites. One of the conclusions from these studies is that some form of local adaptation between host and parasite should exist. Depending on the circumstances, for example, the relative magnitude of host and parasite migration rates among localities, infections by local parasites should be more infective and/or virulent or, alternatively, foreign parasites could be better adapted to the local host (Gandon et al. 1996; Morand et al. 1996). The *Bombus–Crithidia* system belongs to the latter group. In controlled experiments, *C. bombi* from distant areas caused higher levels of mortality than infections with local strains did (Imhoof and Schmid-Hempel 1998b).

Transmission and virulence

The ability to carry out genetic studies with micro-satellite markers of *C. bombi* has suggested that single workers within a colony may harbour several different strains of the parasite, and, consequently, a colony in the field typically becomes infected by a variety of different strains (M. Brown et al., unpublished data). Different strains of *C. bombi* not only differ in their infectivity for workers of different host colonies, but also show variation in the speed at which an infection builds up within the host (Schmid-Hempel and Schmid-Hempel 1993). On one hand, this observation could have some bearing on the social life of the host, especially the question of whether the organization of work in the colony is affected by parasitism. Although bumblebees show only a very weak behavioural profile according to worker age, a worker tends to spend an increasing amount of time away from the nest as it ages. "Fast" strains of *C. bombi* would be more likely to be shed inside the nest and thus would infect nestmates as their next host. "Slow" strains, in contrast, would be more often transferred, via flowers, to other colonies. This maintenance of fast and slow strains due to polymorphism in transmission to different hosts is theoretically possible, but experimental tests have failed to show any evidence for it (Schmid-Hempel and Schmid-Hempel 1993; Schmid-Hempel et al. 1999).

On the other hand, high levels of infection (many parasitic cells present in the host) generally translate into large effects on the host, that is, into parasite virulence. This can be the case in *C. bombi* infections, although the pattern is rather complex. However, for the sake of argument, such a relationship between infection intensity and damage to the host is assumed. Standard theory then assumes that parasites should evolve towards higher virulence if this increases the expected rate of transmission to new hosts (e.g. Bull 1994; Frank 1996). High virulence in *C. bombi* would thus be favoured if it correlated with many infective cells being shed from the current host and thereby increased transmission success to the next host. In *C. bombi*, however, the dose at which the infection is administered only loosely relates to the likelihood that it will become established (P. Schmid-

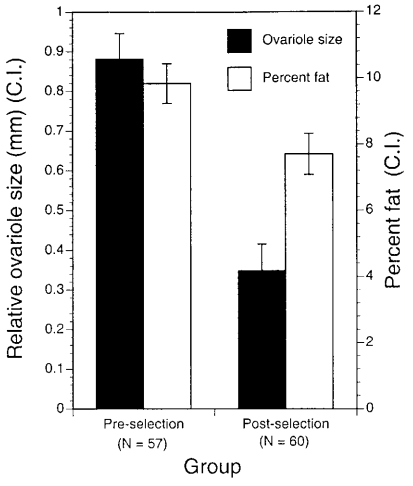

Fig. 3 Adaptation of *Crithidia bombi* to its host, *Bombus terrestris*, in a passage experiment. In the experiment, single workers from healthy colonies were infected with an inoculum of a total of 10,000 cells (extracted and mixed from workers of three infected but independent colonies). The infected workers were then each added to a box of five non-infected nestmates, thus yielding boxes of six workers. Every 4 days half of the workers were randomly replaced by non-infected nestmates, thus forcing the parasite to transmit to new hosts of the same colony. After 16 days and three replacement rounds, the faeces of workers of each box were collected, pooled and an inoculum of 30,000 cells prepared to infect na test workers from the same colony. At this stage, the parasites were considered to have been selected in their respective host colony, i.e. box ("*Post-selection*"). The results of these test infection are shown here and compared with identical infections but with inocula prepared from parasites at the start of the experiment, i.e. before selection ("*Pre-selection*"). The graph shows two measures of host condition: mean (95% C.I.) for relative size of ovarioles (eggs; measured as ratio of length of three largest ovarioles and length of radial cell in forewing), and for the relative size of the fat body (measured as the ratio of dry mass of fat body and dry mass of abdomen). A total of 117 workers from replicates in five experimental colonies were analysed. Selection had a significant effect on the damage done by *C. bombi* [MANOVA with fixed factor treatment (pre- vs. post-selection), for ovariole size: $F(1,107)=277.343$, $P<0.001$; for fat,: $F(1,107)=25.042$, $P<0.001$. Factor colony and the interaction with treatment was significant for ovariole size]

Hempel, unpublished data). In fact, genetic analyses have shown that the total infection intensity in the host correlates with the number of co-infecting strains (Schmid-Hempel et al. 1999). Therefore, the process by which different strains of this parasite infect and establish is different from what the standard models assume. Rather, strains do not appear to intensively compete with each other but instead each has its own “niche” inside the host. How many such “niches” a host offers appears to determine its overall susceptibility and parasite load. According to theoretical analyses by May and Nowak (1995), this kind of infection process leads to different dynamics than that predicted from standard models. In particular, it is expected that different strains of *C. bombi* should occupy a narrow band of virulence close to the value maximizing the parasite's net reproductive rate.

From the natural history of *C. bombi*, we can see that this parasite has many generations and multiplies to large numbers within a worker host. Given short generation times, large numbers, and the presence of different parasite strains within the same worker or colony, it is expected that *C. bombi* could adapt to its prevailing host environment. In particular, when the infection stays within a colony for the entire seasonal cycle, a given parasite line will have to make many passages through the workers of the colony as they are born, live and die. Results from an experiment suggest that *C. bombi* does indeed adapt to its host. More precisely, it appears that virulence increases over time. As shown in Fig. 3, host condition is worse when the host is infected by the parasites that have been passed through a number of workers, as compared to infections by the same parasites prior to selection. According to these results, as the colony cycle unfolds and *C. bombi* is passed on within the colony, the damage done by the parasite will increase and the parasite increasingly becomes more of a threat. This will perhaps force the colony to invest more in defence (see below). Eventually though, the colony cycle comes to an end and reproduction happens before the parasite can adapt any further. However, this also means that offspring queens will become infected by a well-adapted parasite if they acquire it from their natal nest.

Theory furthermore suggests that, in general, serial passage through genetically similar hosts could, at least under certain conditions, lead to an increase in parasite virulence (e.g. Ebert 1998b); passage through genetically different hosts should in turn reduce virulence; a principle that has, in fact, been used in the production of vaccines by attenuation of viruses. The situation is relevant to colonies of social insects, such as *Bombus*, because the host is constrained from adapting. This is because the colony workers all descend from the same mother queen and her mate; the queen never re-mates after colony founding. Therefore, as long as the colony exists, a parasite encounters the same average genetic environment, that is, close relatives of the current host. Experiments to test this prediction have so far produced ambiguous results. Nevertheless, the scenario points to the significance of host genotype for the co-evolutionary dynamics of the system.

The adaptive value of genetic diversification of offspring

Given the genotypic interactions between host and parasite, genotypic diversification of worker offspring within a colony could mitigate against the effects of parasitism. Essentially two processes can lead to such diversification – recombination and multiple mating with different males. In the order Hymenoptera to which *Bombus* belongs, recombination is only effective in the diploid female, whereas the males are haploid. However, the genome-wide recombination rate for *B. terrestris* is not unusually high (Gadau et al. 2000). This shifts the focus to the adaptive value of multiple mating as a possible way to reduce parasite loads in offspring. Indeed, theoretical studies (Sherman et al. 1988; Schmid-Hempel 1994) pro-

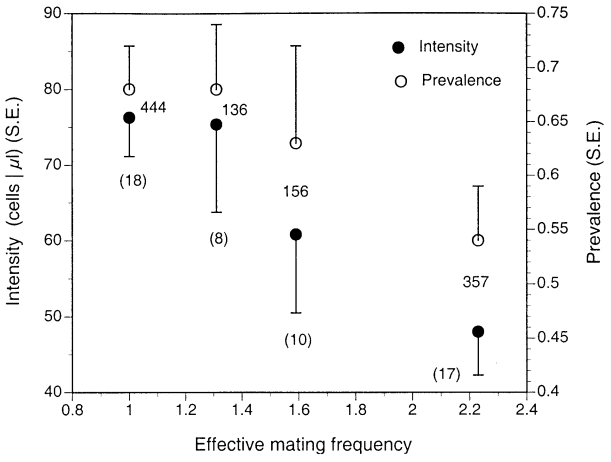

Fig. 4 Intensity (cells/µl of faeces) and prevalence (proportion of workers infested) of infection by *Crithidia bombi* in colonies of *Bombus terrestris*. In this field experiment, four different groups (*A*...*D*) of colonies were compared. The groups differed in the number and kind of inseminations of the queen (*A*: insemination with sperm from a single drone; *B*: mixed sperm from four brothers unrelated to the queen; *C*: mixed sperm from two unrelated males; *D*: mixed sperm from four unrelated males). From genotyping the workers in the resulting colonies with micro-satellites, the paternity for each worker in each colony could be assigned. This was used to calculate the effective mating frequency of each queen (*horizontal axis*). The effective mating frequency is the hypothetical number of males that, if they contributed equally to offspring, would generate the same variance in paternity in the colony as actually observed. Deviations between actual and effective mating frequency result from unequal contributions to offspring by the fathers. In the graph, each group is plotted according to its average effective mating frequency. For both measures, parasitism significantly decreases with an increase in effective mating frequency (which is inversely related to the average within-colony genetic relationship) (ANOVA for ordered alternatives, for intensity $P<0.0001$, and for prevalence $P=0.025$). For clarity, *error bars* are only shown in one direction. *Small numbers in the middle* indicate number of workers checked for the parasite, *numbers in parentheses below* indicate sample sizes (number of colonies in treatment) (after Baer and Schmid-Hempel 2001)

vide evidence that genotypic diversification of offspring as a consequence of multiple mating is beneficial, depending on the costs of mating and parasite pressure in the habitat.

The biology of social insects provides unique opportunities to test such predictions. For example, in contrast to solitary animals, most of the offspring that the queen ever produces stay within the colony to help with raising the brood. Moreover, it is the daughter workers that stay. They have received half of their genes from the queen's mate. If the queen mates with several males, the workers' genotypes in the colony will be diversified accordingly. The consequences of the queen's decision – to mate with one or with several males – will therefore be directly observable by the fate and eventual reproductive performance of the colony. Male offspring, on the other hand, are only produced at the time of colony reproduction and leave the nest. However, they are usually derived from unfertilized eggs of the queen and multiple mating thus cannot affect genotypic variation among them.

Experiments to test this scenario typically suffer from confounding factors. For example, testing females that have naturally mated with many males against females that have mated only once fails to separate the effect of mating frequency from the effect of female condition, where high quality females may achieve many matings and lower quality females only a few. The development of a novel technique to inseminate *Bombus* queens experimentally (Baer and Schmid-Hempel 2000) has made it possible to avoid these confounding factors and to test this scenario precisely. In an experiment, queens were either experimentally inseminated by four unrelated males or inseminated by four brothers; the resulting colonies were either of high or low genetic diversity and were exposed to field conditions. As predicted from the above hypothesis, the high-diversity colonies had lower parasite loads and greater reproductive success (Baer and Schmid-Hempel 1999). The results of this experiment were corroborated by studies of the transmission of *C. bombi* in worker groups of different genotypic diversity (Shykoff and Schmid-Hempel 1991b) and field studies where genotypic variability in the colony was altered by the addition and removal of brood (Liersch and Schmid-Hempel 1998). Recent experimental evidence now suggests that the effect of increased genotypic diversity among colony workers to reduce parasite load is highly repeatable (Fig. 4), but that an adaptive valley in fitness could exist as the female changes from a single to double mating, whereas fitness thereafter increases (Baer and Schmid-Hempel 2001).

Incidental findings

Mating behaviour in B. terrestris

Genetic analyses suggest that the mating frequency of *B. terrestris* females is typically one (Schmid-Hempel and Schmid-Hempel 2000). Apparently, females of *B. terrestris* do not take advantage of the near doubling in fitness associated with multiple mating demonstrated in field experiments (Baer and Schmid-Hempel 1999). This has led to the discovery of male interference with female mating behaviour. In fact, during copulation, which lasts 30–40 min, males of *B. terrestris* transfer a sticky substance from their accessory glands, the “mating plug”. The plug persists for many hours or days (Duvoisin et al. 1999) and its presence prevents re-mating by the queen through a change in female behaviour (Sauter et al. 2001). Further studies have shown that the active substance in the plug is linoleic acid, an unsaturated fatty acid that is very common in insects (Baer et al. 2001). Linoleic acid is extremely effective but it is not known how long this effect lasts, but presumably for hours or a few days. Such time costs are prohibitive for re-mating by the queens under natural conditions. For example, similarly to workers, queens can also become parasitized by conopid flies. Daughter queens thus disappear quickly into hibernation at the time when they are released from

their mother colonies–a time when conopids are very prevalent in the field (Schmid-Hempel et al. 1990). Viewed from the male's perspective, the observed figures suggest that ensuring his paternity by preventing the queen from re-mating (i.e. thus siring all offspring rather than only half if the queen had mated twice) offsets the reduced likelihood (also by one half: Baer and Schmid-Hempel 1999) that his mate heads a successful colony because it has a low genotypic diversity.

Parasites and sociality

Bumblebees are social insects. Therefore, many individuals live together at the nest location. In addition, they are close kin and thus genotypically very similar to one another. From a parasite's perspective, such conditions of high host density and uniform genotypic background are advantageous. We should thus expect the biology of social insects to be heavily affected by parasitism or its avoidance. Whereas the investigation of social parasitism has a long tradition, the investigation of other parasites is relatively recent (Schmid-Hempel 1998).

Parasitism in these systems has a number of obvious ramifications. For example, kin selection theory suggests that hymenopteran societies evolve and are maintained by benefits accumulating for close kin (Hamilton 1964). However, multiple mating occurs and many species are polygynous, which drastically reduces the average genetic relationships in the colony and thus the kin benefits (e.g. Hölldobler and Wilson 1990; Keller 1993; Boomsma and Ratnieks 1996). However, as studies of the *Bombus*–parasite system suggest, close kinship can be both a boon and a bane, because the chance of acquiring a parasitic infection from close kin is higher than that from an unrelated individual (e.g. Shykoff and Schmid-Hempel 1991b). A comparative study across 119 species of ants (Schmid-Hempel and Crozier 1999) showed that high genotypic diversity within colonies indeed correlates with lower parasite loads. Parasitism may therefore explain why social insect colonies are often genotypically more diverse than would be expected from kin selection arguments alone, although a wealth of equally plausible alternative hypotheses exist (e.g. Boomsma and Ratnieks 1996).

Social insect societies are furthermore characterized by a more or less sophisticated organization of work with behavioural and morphological differentiation into various worker castes (Hölldobler and Wilson 1990). In many species, the colony welfare also depends on a single individual – the queen. For example, leaf-cutter ants have very long-lived colonies with several million workers but there is only one queen (Hölldobler and Wilson 1990). Any disease that reaches the queen and kills her is fatal for the entire society. We should therefore expect that the society is organized in a way that minimizes the risk of a pathogen spreading to the “inner circle” of the colony. One possibility is to impose a strict division of labour for individuals that care for and work around the queen, and those involved in work that exposes them to novel infections, for example, the foragers. The contact between these castes should be organized in a way that reduces the transmission of parasites (Schmid-Hempel and Schmid-Hempel 1993; Schmid-Hempel 1998). To what extent parasites select for colony organization is not known but merits further investigations.

A possible Red Queen scenario for bumblebees

The results obtained in our intensive studies on *B. terrestris* and its parasites, especially the trypanosome *C. bombi*, can be synthesized in the following scenario. Some spring queens emerge from hibernation infected with *C. bombi* that they have most probably acquired from their natal nest the previous year. Most queens, however, emerge without this infection. If a queen manages to found a colony, she has a high chance of persisting throughout an entire cycle and reaching the reproductive phase, although with enormously varying reproductive success. In those colonies where the queen carried the infection through hibernation, *C. bombi* readily spreads to the colony workers. As the season progresses, the foragers of both the previously infected and non-infected colonies will pick up novel strains when they visit flowers and carry them back to the nest. However, only a few of these strains will be able to establish within the colony. After some time, the profile of established strains and the resulting infections will vary among colonies within the population. Some colonies have low levels of infection, while other colonies are highly infected.

Although most colonies survive the season, at reproduction only a few colonies manage to produce sexuals, especially daughter queens (Müller and Schmid-Hempel 1993a). It is not yet unambiguously known whether this variation traces back to selection by *C. bombi* over the season, since with the high force of infection in the field, experiments are difficult to carry out. However, increased worker mortality early in the colony cycle could be caused by *C. bombi* infection, combined with the typical adverse environmental conditions of spring. Such early mortality is particularly effective in reducing the reproductive success of a colony (Müller and Schmid-Hempel 1992a; also Schmid-Hempel and Durrer 1991). Recent preliminary evidence, moreover, indicates that selection by *C. bombi* may be particularly strong in the colony-founding phase, with infected queens being much less likely to start a colony than non-infected queens (M. Brown et al., unpublished data). Therefore, granted the working hypothesis that variation in reproductive success is affected by *C. bombi*, the strong genotypic interactions (e.g. Schmid-Hempel et al. 1999) with its host would make sense. For example, the array of genotypes present in the spring queens would be selected against locally by the prevailing strains of *C. bombi* (and possibly other parasites too). With the strong bias in reproductive success (i.e. there are few but highly successful colonies) and highly variable founding success of daughter

queens in the following year (which depends on infection), each year only a few host family lines will dominate the population. The parasite population will accordingly adapt to the prevailing hosts and the most infective strains will become common as the season progresses. This will select against the successful host lines of the previous years (i.e. those that were resistant against the common parasite types then). As host lines are selected against and new host genotypes emerge or immigrate, the parasite population changes accordingly over successive generations, and so on. Overall, we would expect a persistent local turnover of host and parasite types over the generations. This kind of host–parasite co-evolution is known as the "Red Queen"-process (Bell 1982). In this process, parasites continuously adapt to the prevailing host types and hosts continuously escape this adaptation by changing their genotypes, for example, via recombination during sexual reproduction.

Host immune defence

When an infection has occurred, the host's immune system is the last barrier that can prevent fatal damage. Although invertebrates lack the sophistication of vertebrates, insects such as bumblebees have a highly efficient immune system. In fact, the strong genotypic interactions in the bumblebee–parasite relationship with *C. bombi* or *N. bombi* are evidence for an immune defence that is not only selective but in many cases prevents the development of an infection. On the other hand, the fact that infections nevertheless occur suggests that no immune defence is fully effective. One major reason is that the evolution of the immune system is constrained because costs for other fitness components of the organism accumulate (e.g. competitive ability; Kraaijeveld and Godfray 1997). Hence, a perfect defence is simply not affordable. In addition, even if the immune system is in place, its utilization is likely to be costly in terms of energy or nutrients.

Encapsulation is one of the major cellular immune defences in insects. Workers of different colonies vary considerably in their capacity to encapsulate (e.g. Schmid-Hempel and Schmid-Hempel 1998). In two field studies, such colony variation was experimentally balanced and the effect of foraging on the capacity of *B. terrestris* workers to encapsulate a standardized antigen (a nylon implant) was investigated. In both studies, workers that were allowed to forage encapsulated at lower levels than their non-foraging nestmates (König and Schmid-Hempel 1995; Doums and Schmid-Hempel 2000). In the study by Moret and Schmid-Hempel (2000), the logic of the experiment was reversed. The immune system of workers of *B. terrestris* was experimentally induced by elicitors such as LPS (lipopolysaccharides extracted from the surface of *Escherichia coli*) and latex beads. These non-living and non-pathogenic elicitors cannot multiply within the host and are eventually removed by metabolism. However, they induce the immune system to produce anti-bacterial peptides which can be measured with a zone-of-inhibition assay (this is part of the humoral response of the immune system). Under good conditions, that is, when the test animals are fed with nectar, immune-challenged workers easily survive for days and weeks. However, when the animals have no access to food, they will die within 10–20 h. In particular, survival was much shorter (with the mortality rate increasing by more than 50%) when the immune system was induced and the workers had to produce anti-bacterial peptides.

Under natural conditions, most workers are probably permanently immune-challenged by numerous minor infections. Our experiments show that such a permanent challenge is associated with unavoidable costs of immune defence. Moreover, as long as conditions are favourable, these costs can easily be offset by extra resource intake and the effect of parasitism may not be visible. Only when conditions deteriorate do the costs of immunity have a bearing on worker survival.

Parasites and communities of hosts

Many investigators have suspected that parasites may play an important role for the outcome of ecological competition between two host species and therefore for the structure of host communities (Haldane 1949; Freeland 1983; Dobson and Hudson 1986; Price et al. 1986). In central Europe, bumblebees typically form species assemblages comprising 3–5 common species and another 5–10 rare ones. Surveys have demonstrated that all spe-

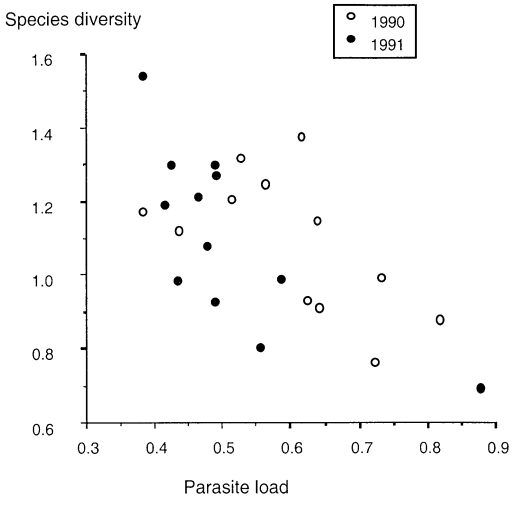

Fig. 5 Species diversity (Shannon-Wiener index, *H*s) in relation to parasite load (the number of parasite species per average worker in the population): stepwise multiple regression identified parasite load as a significant correlate for species diversity in 1990 (coefficient; β=–0.653, r^2=0.362, P=0.039) and 1991 (β=–0.763, r^2=0.542, P=0.006). Bee communities at 12 localities in northwestern Switzerland were sampled along defined transects for 2 years in intervals of two (1990) and three (1991) weeks, respectively. A total of 12 parasite species were identified in the worker samples (conopid flies, parasitic wasps, nematodes, mites, and protozoans). A total of 13 bumblebee species were found. *Each dot* in the graph represents the average over one season for one locality (community) (after (Durrer 1996)

cies in these assemblages are parasitized to various degrees (Schmid-Hempel et al. 1990; Shykoff and Schmid-Hempel 1991a; Durrer and Schmid-Hempel 1995). These surveys also indicate that parasites could perhaps be involved in structuring these communities. For example, when parasite load (the number of parasite species per average worker) is compared to the diversity of the local bumblebee community, a negative relationship emerges (Fig. 5). Such a pattern would be expected when it is more difficult for parasites to invade and persist in diverse host communities. This might apply for parasites that utilize several host species. Alternatively, high parasite loads could force some host species out of the community, thus reducing its overall diversity. Whatever the exact process, the pattern shown in Fig. 5 suggests that parasites and their bumblebee hosts interact in ways that may even have an effect at the community level.

Acknowledgements I thank B. Hölldobler for his encouragement in writing this report. K. Huck, R. Loosli and C. Reber helped in collecting data. M. Brown and R. Schmid-Hempel commented on the manuscript. Some of this work was supported by a grant from the European Union and the Swiss Office for Science and Technology (EERBF MRXCT960072/ BBW 95–0575). I am especially grateful to the Swiss National Science Foundation, whose generous support over many years has made the work reported here possible.

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