Juan Núñez-Farfán
Pedro Luis Valverde *Editors*

Evolutionary Ecology of Plant-Herbivore Interaction

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In the memory of my father (Jesús Núñez
Durán; Nov. 1, 1929–Jan. 12, 2020)
JNF

To Yolotl and Luis Andrés
PLV

Preface

The idea to write an edited book on plant-herbivore interactions, a research area with a young but productive history in Mexico, came across at the Mexican Congress of Ecology, held at León, Guanajuato, in 2017. Most speakers at the symposium gladly accepted to contribute a chapter. Some other colleagues were invited in order to include research topics not covered then. This book gathers the work and ideas of students of interactions between plants with other organisms, but especially with herbivores. Biotic interactions have an utmost importance in phenotypic evolution and in the generation of biodiversity. The role of herbivores as selective agents of a diversity of behavioural, morphological, bio-chemical, physiological, is well established in the specialised literature. The book covers a wide variety of plant traits topics at different levels including populations, communities and ecosystems. Although most chapters deal with plant defence strategies (resistance and tolerance) in different ecological contexts, new avenues of research are examined. These include the role of plant microbiota and herbivores on the interaction, functional plant defence traits, insects as ecosystem engineers of new habitats, differences in defence between sexes in dioecious plants, diversification of herbivores, the evolution of plant defence in stressful edaphic conditions and along environmental gradients, interaction of plants and herbivores in agro-systems, invasive species and the genomics-transcriptomics of plant defence. We hope these investigations will attract the attention of students and researchers interested in evolutionary ecology.

Mexico City, Mexico

Juan Núñez-Farfán
Pedro Luis Valverde

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Pedro Luis Valverde obtained his bachelor's degree in 1990 from the Metropolitan Autonomous University, Campus Iztapalapa (UAMI), in Mexico City. Since 1992 he is a faculty member of the Department of Biology of the UAMI. In 1994, Dr. Valverde obtained his Master of Science in Ecology at the National Autonomous University of Mexico (UNAM). In 2001, he received his Doctorate in Ecology from the Institute of Ecology (UNAM). From 2010 to 2012, Dr. Valverde conducted a research on natural selection on resistance to herbivores in native and introduced range at the Universidad de Sevilla (Spain) with Professor Juan Arroyo. His research interest has been focused mainly to the study of plant-animal interactions and evolutionary ecology of plant defences against herbivorous insects, as well as reproductive biology and ecology of Cactaceae. Dr. Valverde was the President of the Ecological Society of Mexico (SCME), 2018–2020.

Chapter 1

Introduction: Evolutionary Ecology of Plant-Herbivore Interactions

Juan Núñez-Farfán and Pedro Luis Valverde

> *What checks the natural tendency of each species to increase in number is most obscure. …Seedlings, also, are destroyed in vast numbers by various enemies; …on a piece of ground…I marked all the seedlings of our native weeds as they came up, and out of 357 no less than 295 were destroyed chiefly by slugs and insects.*
>
> C.R. Darwin (1859, Chap. 3)

Abstract The interaction between plants and their herbivores has spurred the evolution of multiple traits in the resource base – the host plants – as well as the evolution and diversification of different feeding habits of their consumers. This volume examines the extent to which these relationships have led to the microevolution of plant defence traits (i.e., resistance and tolerance), diversification of herbivores' feeding habits and radiation of plant and herbivore species, host plant-herbivore associations, patterns of defence at the scale of plant communities and environmental gradients, as well as disruption of plant-herbivore interaction patterns in human-modified environments (e.g., those affected by the loss or decline of herbivores, invasive species, land use change, or even climatic change). The book examines our current understanding of these relationships and the future directions that warrant further work and hold a promise in this important field in light of current and future anthropogenic impact.

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Keywords Adaptive evolution · Chemical and physical defences · Coevolution · Human-induced effects on biotic interactions · Macroevolution · Natural selection · Plant resistance · Plant tolerance

The consumption of plant live tissues by animals (Crawley 1983; Strauss and Zangerl 2002; Del Val 2012), herbivory, is an interaction that started, at least, from the Middle Devonian (ca. 385 Mya) (Labandeira and Currano 2013). Nowadays, there is a consensus that many anti-herbivore adaptations of plants, and counter adaptations by herbivores, are the evolutionary result of *the* interaction (Thompson 2005). For instance, it is believed that the great variation of chemical and physical phenotypes of plants and herbivores constitutes a result of reciprocal responses in what has been termed an “arm race” between plants and herbivores (Ehrlich and Raven 1964; Rausher 2001). Furthermore, the interaction between plants and herbivores is thought to have promoted not only the origin of many adaptations but also speciation and diversification of the interactors (Thompson 1994, 2005; Strauss and Zangerl 2002).

Besides the evolutionary consequences at the population level, herbivory is involved in many ecological contemporaneous processes, giving rise to eco-evolutionary dynamics. Likewise, herbivory can affect community diversity and energy fluxes in ecosystems (see review by Strauss and Zangerl 2002). Humans, too, have altered plant-herbivore interactions and their evolution, by modifying genetic variation of plants (improved crops, transgenics, herbicides) (Dirzo and Raven 2003), changing selection pressures on herbivores and their enemies (proliferation of and resistance to pesticides) (Despres et al. 2007), or decimating them (defaunation) (Chap. 12; Dirzo et al. 2014). Considering a phytocentric perspective first, it has been recognized that plant defence against herbivores includes two complex strategies, resistance and tolerance (Núñez-Farfán et al. 2007), as we discuss next.

Plant Defence Characters: Resistance

Darwin (1859) clearly thought that herbivores would exert selection on plants when attacked and, in some instances, killed them. Herbivores were thought to exert selection on plants as they are a biotic factor of plants’ environment and are in this environment one facet that spurs adaptation. It is less clear, however, when scientists began to search by for an explanation for the responses of plants to prevent damage, and death, from herbivores and in turn how herbivores can cope and surpass the “barriers” erected by plants. Historically, each member of the interaction was studied, mostly, by botanists and entomologists in an independent way (perhaps owing to the division of botany and entomology or zoology departments among universities).

Plant resistance against herbivores is the ability of a plant genotype to prevent or to reduce the level of damage exerted by its enemies (Painter 1958; Núñez-Farfán et al. 2007; Stenberg and Muola 2017). Plant resistance to herbivores includes different plant traits. In general, resistance of plants to herbivores includes chemical and structural traits. The former, secondary chemical compounds, a salient character of plant groups and not linked before with primary plant functions, are now widely recognized as a main "barrier" to deter, harm or kill herbivores and to reduce fitness losses by them. The latter include spinescence, pubescence, sclerophylly and deposits of minerals in plant tissues (Hanley et al. 2007).

It was Fraenkel (1959) who established explicitly the hypothesis that secondary plant metabolites *mediate* the interactions between plants and herbivores, particularly insects. He proposed that (1) secondary metabolites play a key role in the selection of host plants by insects, functioning either as repellent (providing resistance to plants) or attractant. (2) The great diversity of secondary compounds equals the diversity of interactions between plants and herbivores. (3) Secondary metabolites play no role in primary functions of plants and herbivores but trigger substances that induce or prevent the intake of nutrients from plants by herbivores. (4) In contrast with primary nutrients, secondary metabolites possess odour and taste that provokes reactions to the food by insects. Since plant species differ less in primary nutrients than in secondary metabolites, insects should be capable of developing many plant species which is not true; secondary metabolites are restricted to phylogenetic groups of plants (Fraenkel 1959).

Many secondary compounds have been identified amounting more than 200,000, terpenoids and alkaloids being the more abundant (Table 1.1; Mithöfer and Boland 2012). The effect of secondary metabolites on animals includes, among other specific effects, the disruption of cell membranes, inhibition of transport of ions and nutrients, signal transduction, metabolism as well as disruption of hormonal control or

Table 1.1 Types of plant secondary metabolites and the approximate number of known compounds within them

Compounds	Approx. number of compounds known
Terpenoids	>30,000
Steroids	ca. 200
Cardenolides	ca. 200
Alkaloids	>12,000
Fatty acid derivatives	Not determined
Glucosinolates	ca. 150
Cyanogenic glucosides	ca. 60
Phenolics	>9000
Polypeptides	Not determined
Nonprotein amino acids	>200
Silica	1
Latex	Variable composition

Modified from Mithöfer and Boland (2012) physiological processes (Mithöfer and Boland 2012). For instance, in the case of latex, given its sticky nature, it can entrap insects preventing feeding (see Becerra and Venable 1990), whereas alkaloids can affect the nervous system and reduce growth and survival of insects (Krug and Proksch 1993). In *Datura stramonium* (Solanaceae), the concentration of the tropane alkaloid scopolamine reduces the intensity of seed predation by the weevil *Trichobaris soror* (Miranda-Pérez et al. 2016).

Spinescence, pubescence, sclerophylly and mineral deposition are considered structural, physical, plant defence traits (Table 1.2). These traits probably have a dual or multiple functions in plants. Perhaps their primary role was not defence. Furthermore, pubescence (glandular trichomes) and mineral deposition may function also as chemical defence. In the case of spines and trichomes (see Chap. 2), although both structures may reduce radiation flux, their role as defence has been demonstrated. Spines often offer protection against feeding by vertebrates, but, as in the case of cacti, spines do not protect plants against insect herbivores (see Chap. 6). In contrast, glandular and nonglandular trichomes are more effective against predation by invertebrates, notably insects. Recent studies demonstrate that the within population variation in the frequency of glandular ("sticky") and nonglandular ("velvety"), trichomes in *Datura wrightii* are maintained by negative frequency-dependent selection and that the specialization of two herbivores, each feed preferentially in one morph, may control the frequency of the "velvety" and "sticky" morphs across populations (Goldberg et al. 2020).

Sclerophylly, hard leaves, is considered to have different functions in plants, including leaf support, resistance to wilting, heat, cold or protection against UV radiation, among others (Hanley et al. 2007). However, reducing palatability and digestibility also may reduce leaf tissue losses by herbivory. Thus, the defence role of sclerophylly may constitute a by-product of selection of other functions. But evidence indicates that sclerophylly reduces damage by herbivores. For instance, the analysis of leaf toughness in deciduous and evergreen plant species in a dry tropical forest revealed that the latter had tougher leaves than deciduous species and that leaf toughness correlated negatively with the amount of herbivory (Pringle et al. 2011).

Deposition of minerals, mainly silica, in leaves and stem tissues is thought to function as structural support (in grasses) enhancing plant growth (Hanley et al. 2007). Yet, like sclerophylly, mineral deposition on plant tissues increases toughness and then increases resistance to herbivores' damage (Hanley et al. 2007). It has been hypothesized, for instance, that the evolution of grasses and large mammalian grazers during the Eocene (45–55 Mya) has been tightly connected, involving the evolution of silica bodies on leaf epidermis and hypsodont dentition (high-crowned teeth),

Table 1.2 Structural plant defence characters (from Hanley et al. 2007)

Structural defence trait	Types
Spinescence	Spines, thorns, prickles
Pubescence	Glandular and nonglandular trichomes
Sclerophylly	Though leaves
Minerals	Silica, Calcium

respectively (Simpson 1951; Stebbins 1981). An outstanding experimental study conducted by McNaughton and Tarrants (1983) demonstrated the link between grass leaf silicification and historical herbivory pressure in the Serengeti. In factorial experiment, they measured the content of silica in leaf blades of three species of grasses from two locations differing in the intensity of grazing (heavy and light). The species were grown in a common garden for 4 years, with and without clipping and with and without soluble silica. Results revealed that the plants from the more heavily grazed site accumulated more silica than plants from the less heavily grazed site. Silica accumulation was higher in clipped plants indicating inducibility of silicification. The results suggest that continuous selection of silica content of plants has produced differentiation between plants of sites with different histories of herbivore pressure.

Plant Defence Characters: Tolerance

As defined by Painter (1958), tolerance to damage by herbivores implies the survival of plants under "levels of infestation that would kill or severely injure susceptible plants" and "involves the general vigour of the plant" (Painter 1958). That is to say, plant tolerance responses are aimed to maintain plant fitness under the attack of herbivores (Rosenthal and Kotanen 1994; Strauss and Agrawal 1999; Stowe et al. 2000; Núñez-Farfán et al. 2007). Nowadays, tolerance can be envisaged as a pattern (i.e., plasticity) and as a mechanism to maintain plant fitness in the presence of damage (see Chap. 4).

Several plants' traits are involved in the tolerance response to damage (see Chaps. 3 and 4). Stowe et al. (2000) have revised the evidence of traits known to provide tolerance to damage grouped in two categories: resource allocation and plant architecture. Patterns of resource allocation include relative allocation to growth, reproduction and storage, qualitative characteristics of plant organs, morphological and physiological and the ontogenetic stage at which allocation occurs (Boege and Marquis 2005). Patterns of architecture may influence resource capture and plant modularity. As pointed out by Stowe et al. (2000), plant resource allocation and plant architecture patterns are interrelated. Recently, the potential role of increased gene expression associated with overcompensation following meristem damage may be linked to plant tolerance to herbivory (see Chap. 3).

Microevolution of Plant Defence Against Herbivores: Resistance and Tolerance

The evolution of plant defence in response to the attack of herbivores implies that the latter affects plant reproductive success. However, the evolutionary response is only possible if individual variation in defence traits possesses genetic variance in the population (Endler 1987). The first studies of selection of plant defence to herbivores were made in polymorphic traits (e.g., Jones 1962, Dirzo and Harper 1982, Kakes 1989). A major advancement in the study of adaptive plant evolution of defence was the incorporation of quantitative genetics and the measurement of natural selection in correlated characters (Lande and Arnold 1983; see review by Berenbaum 2001). The first two studies that assessed natural selection of plant defence and genetic variance in quantitative traits were conducted in two wild plant species. Berenbaum et al. (1986) demonstrated in the system *Pastinaca sativa* and its herbivore *Depressaria pastinacella*, the effect of natural selection on the concentration of four furanocoumarins. Likewise, Rausher and Simms (1989) detected natural selection on resistance to damage by herbivores in *Ipomoea purpurea* and *Datura stramonium* (Núñez-Farfán and Dirzo 1994) and on defence traits (glucosinolates and trichome density) in *Arabidopsis thaliana* (Mauricio and Rausher 1997). The relevance of these studies resides in that it was possible to test the assumptions, at the microevolutionary level, of the coevolution between plants and herbivores (cf. Ehrlich and Raven 1964) that may promote "escape and radiation."

Few years later, it was proposed that the simultaneous allocation of resources to resistance and tolerance to pathogens (Simms and Triplett 1994) and/or herbivores (Fineblum and Rausher 1995) may involve costs and hence a trade-off between strategies. This would imply that natural selection will not favour both strategies (Mauricio et al. 1997). Models indicate that mixed strategies of plant defence could evolve simultaneously (Fornoni et al. 2004). In addition, natural selection may favour traits that confer resistance and tolerance (Stowe et al. 2000; Stowe 2013). Until recently, it has been also proposed that the analysis of resistance and tolerance in the interaction between animal and their enemies (pathogens) could help better understand their coevolutionary dynamics (Svensson and Råberg 2010).

Macroevolutionary Studies of Plant Defence Against Herbivores

The studies above the species level revealed that either diffuse or strict, the historical interaction between plant and herbivores must produce reciprocal evolution of traits that mediate the interaction (i.e., chemical and physical defences). That is to say, the interactors affect their fitness reciprocally (Janzen 1980; Oyama 2012). After the proposal of Ehrlich and Raven (1964) of coevolution between plants and butterflies, evolutionary biologists involved themselves to study the process, natural selection, responsible in generating the patterns of host plant-herbivore association and the phenotypic diversity of characters that mediate the interactions (Thompson 2005). However, micro- and macroevolutionary studies followed different routes until the incorporation of the phylogenetic and comparative methods to the study of co-adaptive evolution. Nowadays, the evidence of coevolutionary interactions between plants and herbivores, given rise to a diversity of plant defence and animal counter defence is outstanding (see a whole account in Kariñho-Betancourt 2018, 2019).

The Scope of This Book

The study of plant-herbivore interactions has expanded explosively over the last years. This is due to the search for a better understanding of the mechanisms and patterns that govern and result from the most ubiquitous interaction in nature, plants and herbivores. This is reflected in the amazing growth of specialized journals that publish research on plant-herbivore interactions from different perspectives, methodological tools, theoretical and empirical approaches, levels of organization (from individuals to ecosystems), landscape and conservation focused, among others. This book reflects such diversity of studies, addressing the complexity of evolutionary ecology of plant-herbivore interactions.

Contributions in this book have been integrated into four sections: the evolution of plant defence (microevolution), community ecology of interactions, phylogeny (macroevolution) and genomics. Yet, it must be recognized that this arrangement is arbitrary as many chapters can fit in more than one section and that other fundamental areas are not covered in this book. All contributions offer open questions or perspectives that merit further research.

One of the salient features of the relationships between plants and herbivores relates to the fact that some plants, either individuals, sexes or species in a community, are more intensively eaten than others. This omnipresent fact in nature has promoted the search of the reasons of this. Ecologists have recognized that plants and animals have evolved many adaptations and counteradaptations as a result of such an interaction. For instance, plant trichomes, glandular and nonglandular, constitute the frontline of defence against damage by herbivores. Kaur and Kariyat (Chap. 2) have revised the biology of plant trichomes including not only their defence role but also their role in the face of abiotic stressors. They expose different lines of research on trichomes, from basic and applied ecological studies, as well as molecular ones aiming to detect the expression and quantification of genes associated with trichome biosynthesis and biochemistry.

Although plant tolerance to herbivores has been recognized for a long time, it was not considered a defence strategy until recently. In this book, two chapters deal with the study of the unifying mechanisms responsible for plant tolerance (Chap. 4) and resource allocation and plant defence in wild and model plants (Chap. 3). Also, the adaptive evolution of plant defence, including tolerance and resistance, in native and non-native habitats is reviewed (Chap. 5). As in natural populations, non-native and sometimes invasive, plant populations fulfil the conditions for adaptive evolution (Chap. 5). However, there is scarcity of attempts to quantify natural selection on plant defence in introduced plant populations. In this chapter, some predictions on the evolution of defence in relation to the nature (i.e., generalist and specialist) of herbivores are made (Chap. 5).

Plants and their herbivores, however, do not exist in a biodiversity vacuum. Their communities include multiple other interactors, competitors, mutualists, parasites, etc., altering their eco-evolutionary dynamics. In Chap. 6, besides reviewing the evidence of herbivory in the paradigmatic Cactaceae family, the authors show a complex interaction between *Myrtillocactus geometrizans*, two competing species of herbivores, one mutualistic ant to one herbivore and herbivores' enemies. The detrimental effect of one herbivore on plant fitness may depend on the presence of its mutualistic partner. This study exemplifies the complexity of plant-herbivore interactions in a community context.

It seems that the interactions between plants and herbivores occur under a "microbial milieu," scarcely explored until now, although such interactions may be common. Plants' endomicrobiota can play a role affecting herbivores, and, in turn, insects' microbiota can affect plant defence. In Chap. 7, the potential feedbacks between the endomicrobiota of plants and insects and their role in the interaction are examined, together with current theoretical approaches for their study.

Organisms do not suffer their environment passively. The evidence of ecosystem engineering by insect herbivores is plentiful, as reviewed in Chap. 8. Here, the author traces the origin and diversity of the insect herbivore groups acting as ecosystem engineers and the strategies to create new environments. Also, the author reviewed the communities of arthropods associated with habitats modified by different guilds of insect herbivores.

Dioecy, the presence of separate female and male individuals within a species, although very common in animals, is also present in some plant species. Due to differences in resource allocation between sexes to different functions (i.e., growth, reproduction, defence, etc.), it has been hypothesized that herbivores may exert a different impact on the fitness of male and female plants. In Chap. 9, the authors review the evidence and discuss how differences in defence against herbivores may affect higher trophic levels.

The communities of plants are integrated by different functional groups. Such groups may interact with herbivores in different ways or deploying different defences to prevent damage by herbivores. In Chap. 10, the authors offer an approach to study plant-herbivore interactions in natural communities, linking plant functional groups with plant syndromes of defence against herbivores – suites of characters including physical, chemical and nutritional reducers, phenology, etc. – in order to understand the evolution of anti-herbivory syndromes at the community level.

Herbivores can cope with man-modified environments where the abundance of their natural host plants is changed. Such is the case of landscape and farming practices in agroecosystems that can impact herbivore regulation. In Chap. 11, the authors analysed the management of practices that affect biodiversity either positively or negatively. They illustrated this in a study case with a tropical fruit crop and proposed the use of structural equation modelling, revealing that intensive management could affect the third trophic level.

It is an established fact that plants can evolve chemical, structural and biotic "weapons" to defend themselves from herbivores. However, can plants cope with herbivores in stressful environments? This is less clear. In Chap. 13, the authors tackle this question in relation to harsh, bare, edaphic environments. They do this, by integrating phylogenetic and ecological studies in the diverse clade *Streptanthus* (Brassicaceae). Besides reviewing evidence on how plants cope with herbivores in these environments, they outline the selection constraints for the evolution of plant defence in harsh habitats. The chapter advances new avenues of research of plant defence evolution in stressful environments.

Contemporary interactions between plants and herbivores are the result of historical, evolutionary associations, producing diversification and extinction of interactors. In Chaps. 14 and 15, the authors analyse the phylogeny of specialist and generalist insect herbivores. In Chap. 14, the author analyses plant-herbivore association between the seed specialist predators of the genus *Trichobaris* (Coleoptera: Curculionidae) with solanaceous plant species but in particular with species of the genus *Datura*. On the other hand, Chap. 15 documents the diversification of a group of generalist herbivores of the genus *Sphenarium* (Orthoptera: Pyrgomorphidae). Also, the mechanisms of speciation in insects are discussed. A complementary perspective to the book's chapters focuses on insects as herbivores; a related chapter examines the ecological interactions between plants and vertebrate herbivores in light of the current anthropogenic impact on animal life ("defaunation"), including a description of our current understanding of this phenomenon and the consequences thereof for plants at the level of populations and the overall community (Chap. 12).

Recent advancements in the study of plant-herbivore interactions have incorporated genomic tools. Chapter 16 reviews the evidence of the evolution of plant defence from the genomic perspective, namely, the identification of genome regions involved in the expression of constitutive and induced plant defence. Studies have revealed the basic mechanisms of signalling and response to damage. Genomic studies may also contribute to understand the links between tolerance responses and resistance (see also Chap. 3). A beautiful example of the genomic approach to study the interaction between plants and herbivores is illustrated by gall-forming insects (Chap. 17). In this chapter, transcriptomic analyses evaluated the gene expression patterns during the development of galls in the oak tree *Quercus castanea* induced by the wasp *Amphibolips michoacaensis* (Hymenoptera), relating this with herbivore larval development.

The complexity of the nature of the interactions between plants and herbivores is illustrated by the closing chapter (Chap. 18). Here, the authors analysed environmental gradients, altitudinal and latitudinal, to study plant defence and herbivory in temperate trees. These systems offer the opportunity to test patterns for cline variation of plant defence and its genetic basis, using genomic tools. Their results revealed striking patterns that are discussed. This integrative chapter calls for comprehensive studies incorporating landscape genetics and genomics, to tackle questions at micro- and macroevolutionary levels.

Collectively, the chapters that comprise this volume make it abundantly clear that biotic interactions such as those between herbivores and plants represent an omnipresent, dynamic engine of plant and animal speciation and consequently a critical factor for the genesis and maintenance of the biodiversity that characterizes the planet and supports human wellbeing.

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Part I
The Evolution of Plant Defence

Chapter 2
Role of Trichomes in Plant Stress Biology

Jasleen Kaur and Rupesh Kariyat

Abstract In this chapter, we will examine the role of trichomes in plant stress biology. Trichomes have been long studied as the first line of defense against herbivores. We review the studies that have examined the structure, classification, and diversity of trichomes in flowering plants, followed by their modes of action against abiotic and biotic stressors. We also review the studies on herbivore X trichome interactions and how trichomes play a role in both direct and indirect plant defenses. Next, we use results from our research to highlight the importance of trichomes as an effective measure of plant defense and how genetic variation affects trichome-mediated defenses. And, finally we propose some exciting new areas of research on trichomes and trichome-mediated defenses for future work.

Keywords Defense · Glandular trichomes · Herbivores · Nonglandular trichomes · Plant stress · Plant trichomes

Plants, being sessile living organisms, are constantly exposed to unfavorable abiotic and biotic factors that exert significant selection pressures on them (Mithöfer and Boland 2012). Various abiotic stressors include extreme temperature, drought and salinity (Kaur et al. 2008; Thakur et al. 2010; Ashraf et al. 2018), whereas biotic stressors mainly include pathogen and insect herbivory (Atkinson and Urwin 2012). While the biotic stressors directly attack the host plant, the abiotic stressors, in addition to affecting their growth and development, also render them weak and predispose them to be attacked by biotic stressors. However, despite the absence of a brain or a central nervous system, plants have evolved a suite of coordinated defenses to tolerate/protect themselves against both biotic and abiotic stressors (Singh et al. 2016). They employ a wide gamut of constitutive (preformed) and induced defenses (induced upon herbivory/pathogen infection) in response to these stressors (Howe and Jander 2008; Kariyat et al. 2012a, b and 2013). More specifically, both

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constitutive and induced defenses can either be direct or indirect (Howe and Jander 2008; Kariyat et al. 2012a, b and 2013), in their mode of action. Direct defenses include the innate ability of the plants to counter/offset herbivory using morphological structures like spines, trichomes, thorns, thicker epidermis, and waxy cuticle or by the production of defensive biochemicals like alkaloids, tannins, phenols, anthocyanins, etc. that affect herbivore growth and development (Hanley et al. 2007; Hauser 2014; Kariyat et al. 2017, 2019). On the other hand, indirect defenses include the ability of plants to attract natural enemies like predators and parasitoids by the emission of a unique blend of volatiles known as herbivore-induced plant volatiles (HIPVs) (De Moraes et al. 1998; Pichersky et al. 2006; Mccormick et al. 2012; Aljbory and Chen 2017) or through extrafloral nectaries (Heil et al. 2001). Among these different types of defenses, trichomes are commonly considered as the first line of defense and can protect the plants by both physical and chemical means (Shanower 2008). Trichomes are the epidermal cells that differentiate into hairlike protuberances on the aerial plant organs (Marks 1997; Glas et al. 2012; Kariyat et al. 2013) and armor the immobile plants against biotic stressors like herbivores' feeding and oviposition, and also against other abiotic stressors like extreme low and high temperatures, excessive light intensity, drought, and even harmful ultraviolet (UV) radiation (Kaur et al. 2008; Shanower 2008; Thakur et al. 2010; Burrows et al. 2013; Ashraf et al. 2018).

Classification and Synthesis

Trichomes are found in a diverse array of shapes and structures (Xiao et al. 2017). They can be broadly classified into nonglandular (Fig. 2.1) and glandular trichomes (Fig. 2.2) and could be either unicellular or multicellular (Wagner et al. 2004). Nonglandular trichomes are usually unicellular and are found on the majority of the angiosperms, few bryophytes, and gymnosperms (Werker 2000). For example, both thale cress (*Arabidopsis thaliana*; family Brassicaceae) and cotton (*Gossypium* spp.; family Malvaceae) possess only simple nonglandular trichomes (Mathur and Chua 2000; Glas et al. 2012). However, trichomes in cotton are unbranched (Fig. 2.1a) and more pronounced on seed surface, while the trichomes in *Arabidopsis* sp. could be either unbranched (Fig. 2.1a) or branched (also known as stellate and multicellular) (Fig. 2.1b), and can be produced on any aerial plant part (Kim and Triplett 2001; Wang et al. 2004).

Glandular trichomes (Fig. 2.2), on the other hand, are usually multicellular and have well-differentiated basal, stalk and apical cells. Approximately 30% of the vascular plants possess glandular trichomes. For example, plants of plant families Lamiaceae (*Sage*, *Salvia divinorum*; mint, *Mentha x piperita* etc.) and Solanaceae (Tomato, *Solanum lycopersicum;* potato, *S. tuberosum;* Tobacco, *Nicotiana tabacum*) have dense glandular trichomes (Fahn 2000; Glas et al. 2012). Furthermore, glandular trichomes can be characterized into two main classes: peltate and capitate trichomes. For example, capitate trichomes are encountered in plants of the families

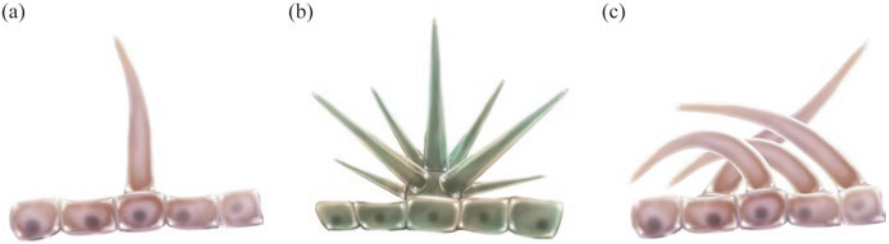

Fig. 2.1 Schematic representation of the structure of nonglandular trichomes: (**a**) simple unbranched, sharp and pointed trichomes; (**b**) branched (stellate) trichomes with arms arranged into a characteristic starlike shape surrounded by few epidermal cells on a vertical spike; and (**c**) hooked pointed trichomes with a prominent curve at their tip, usually aligned at an angle between 20 and 90 degrees to the leaf epidermis (Credits: Annette Diaz)

Fig. 2.2 Schematic representation of the structure of glandular trichomes: multicellular trichomes consisting of secretary head supported on pedestal made of vacuolated basal cell and stalk cell. The secretary head contains secondary metabolites (Credits: Annette Diaz)

Solanaceae and some Lamiaceae (e.g., *Salvia* sp.), while peltate trichomes can be found in some other members of Lamiaceae family like peppermint (*M. piperita*) and basil (*Ocimum basilicum*). The major difference between these two trichome types is in the production and storage location of the secondary metabolites in the trichome structure. Peltate trichomes utilize the subcuticular cavity to produce and synthesize volatile secondary metabolites, whereas capitate trichomes usually synthesize and store their secondary metabolites in the terminal cells and/or at the stalk tip (Gerd et al. 2014).

Various histochemical studies suggest that secretions of peltate trichomes mainly comprise of terpenoids (e.g., wild dagga, *Leonotis leonurus*; peppermint, *M. piperita*), and those of capitate trichomes consist of mostly polysaccharides, proteins and lipids (Werker et al. 1985; Ascensão et al. 1997; Ascensão and Pais 1998). However, small amounts of terpenoids can also be found in capitate trichomes of some plants. For instance, *M. piperita* capitate trichomes can contain monoterpene traces in their essential oil secretions (Amelunxen et al. 1996; Ascensão et al. 1997), and *L. leonurus* trichomes can also contain meager quantities of flavonoids (Ascensão and Pais 1998). However, it had been emphasized that peltate glands are responsible for a major portion of terpene production in peppermint (Turner et al. 2000). Also, the secretions of capitate trichomes are mostly nonvolatile compounds, while those of peltate trichomes are mostly volatile compounds (Tissier 2012). For example, geraniol is contained in the peltate trichomes of lemon basil, which after oxidization produces the aldehyde geranial. Further, geranial undergoes keto-enol tautomerization to form neral. Together, the mixture of the new compound neral and the aldehyde geranial is known as citral, responsible for the lemony flavor of the plant. Another member of Solanaceae, tobacco (*N. tabacum*, family Solanaceae) trichomes, has been found to contain defense compounds such as cembratrieneols and cembratrienediols. Furthermore, a hallucinogen terpene called salvinorin, is contained in *Salvia divinorum*. Recently, antimalarial properties have been studied for the sesquiterpene secretion called artemisinin in the trichomes of *Artemisia annua* (family Asteraceae) (Schilmiller et al. 2008).

Structurally, stalk length in capitate trichomes surpasses half the height of the secretory head, while peltate trichomes are shorter (Abu-Asab and Cantino 1987; Ascensão and Pais 1998). However, the capitate trichomes exist in varied morphologies with respect to stalk length and their secretory head and further classified into different types (Werker et al. 1985; Ascensão and Pais 1998). For instance, by the virtue of electron microscopy, type 1 and type 2 capitate trichomes were identified in *L. leonurus* such that type 1 trichomes posessed short cylindrical stalk and type 2 possessed long conical stalk (Ascensão and Pais 1998).

Also, peltate trichomes have more variability than the capitate trichomes with respect to the form of the stalk cells and the number of cells on the secretory head and surrounding cells. Studies have described peltate trichomes to possess distinct subcuticular storage cavity resting on a pedestal made up of vacuolated basal cell, stalk cell, and eight-celled apical glandular disc (Fahn 1979; Maffei et al. 1989; Brun et al. 1991; Ascensão et al. 1997; Turner et al. 2000; Camina et al. 2018). Peltate trichomes also have a globular shape owing to the acquisition of secretions in the apical cavity. On the contrary, capitate trichomes are usually four celled but with varied morphologies. For instance, *M. piperita* has both capitate and peltate structures where capitate hair consists of the circular head having four cells and a stalk (Werker et al. 1985; Gonçalves et al. 2010).

Trichome-rich species of wild (*Solanum pennellii*, *S. carolinense*, *S. habrochaites*, and *S. peruvianum*) and cultivated *Solanum* (*S. lycopersicum*) have been extensively studied in the past owing to their importance in plant resistance against herbivory and also for the presence of nonglandular, glandular, and even both in some cases (Kariyat et al. 2013, 2017, 2018, 2019). In 2012, Glas et al. have redescribed the trichome morphology in wild and cultivated *Solanum* spp. Out of the total eight trichome types previously described by Luckwill (1943) and revised by Channarayappa et al. (1992), they have clarified the classification as follows: types

I, IV, VI, and VII are glandular trichomes, and types II, III, V, and VIII are simple nonglandular trichome types (Luckwill 1943; Channarayappa et al. 1992; Glas et al. 2012). Of the glandular trichomes, type I and IV are both described as capitate with differences in their length. Type I trichomes are significantly longer when compared to type IV. Both type I and IV trichomes are predominant in wild *Solanum* species like *S. habrochaites*. However, in the cultivated tomato species, type I is rarely found and type IV is completely absent. Interestingly, type VI is predominant in both wild and cultivated tomato species (Glas et al. 2012). The differences among these types are mainly due to the variation in the number of stalk cells and secretory cells. Also, these trichome types differ in genes and transcripts responsible for the synthesis of different biochemicals that they harbor (Glas et al. 2012).

At the functional level, nonglandular trichomes (Fig. 2.1) act as structural defenses, physically affecting insect herbivores by restricting their access to the leaf epidermis, causing physical entrapment or inflicting injury to them (Dalin et al. 2008; Peiffer et al. 2009; Weinhold and Baldwin 2011; Szyndler et al. 2013; Weigend et al. 2017). On the other hand, glandular trichomes (Fig. 2.2) assist in herbivore defense as a chemical means or as a combination of both physical and chemical defenses. This is primarily accomplished by storing and injecting toxins and/or signaling molecules that either directly reduce herbivore feeding or in some cases lead to multi-trophic interactions (Peiffer et al. 2009; Weinhold and Baldwin 2011). Usually, they contribute to direct toxicity in insects, entrapping them by the production of sticky exudates and anti-nutrition factors, and inducing anti-herbivore defense signaling in the host plant – primarily mediated through the phytohormone jasmonic acid (Peiffer et al. 2009; Kariyat et al. 2017).

Although a significant amount of research has been dedicated to understanding trichome-mediated effects in various systems, we still lack a detailed understanding of trichome development. Studies in the model plant *A. thaliana*, which possesses only nonglandular trichomes (Schnittger and Hülskamp 2002), suggest that unicellular trichomes in this plant emerge from the primary meristem of the plant epidermis (Fig. 2.3), by dividing mitotically. In the process, several epidermal cells surrounding the germinating trichomes divide continuously and simultaneously.

Fig. 2.3 Schematic representation of initiation of trichome formation: unicellular cell germinating from the leaf epidermis is illustrated. Consequently, this small protuberance enters mitosis and then endoreduplication cycle. (Credits: Annette Diaz)

Consequently, trichome cells enter the endoreduplication cycle, where DNA replication takes place without any nuclear and cellular divisions. Usually, this cycle is accomplished before trichome outgrowth on the surface. And, trichome cell alters its polarity and starts emerging as an outgrowth (first branching event). Simultaneously, the second endoreduplication cycle is initiated, followed by the second branching event. The third endoreduplication and the second branching events take place concomitantly. While the fourth (last) endoreduplication cycle is ongoing, trichome cell undergoes expansion, yielding approximately 0.5-mm-long mature tri-branched/unbranched trichomes (Marks and Feldmann 1989; Schnittger and Hülskamp 2002) perpendicular to the plant surface (Marks and Feldmann 1989). Despite detailed studies of the trichome types and their characterization, we have limited knowledge of the synthesis of glandular trichomes.

Trichome formation is a complex process, and one simple model is not enough to explain their formation, patterning, and development through the different growth stages. Different phytohormone pathways have been found to be responsible for the regulation of trichome development (An et al. 2011). For example, gibberellins, cytokinins, jasmonic acid, and salicylic acid have been shown to regulate trichome initiation (An et al. 2011). However, their interplay and potential feedbacks largely remain unclear. Among these, jasmonic acid (JA) is considered as the key signaling molecule responsible for trichome production and induction. For instance, JA and JA-Ile have been found to induce resistance to herbivory by inducing trichomes in *Arabidopsis* spp. (Traw and Bergelson 2003; Li et al. 2004; Boughton et al. 2005; Peiffer et al. 2009; An et al. 2011). It has been recently demonstrated that MYB-bHLH-WDR (MBW) complex can modulate the gene expression of *GL1 (GLABROUS 1)* and *GL3 (GLABROUS 3)*, successfully resulting in the trichome induction in the wild type of *Arabidopsis* spp. but compromised in the JA mutant *coi1–coi2*. In the absence of JA, JA-ZIM domain (JAZ) proteins (present in plant cells) can physically bind to *GL1* and *GL3*, which inhibits the formation of MYB complex, clearly showing that JAZ proteins can attenuate JA signaling. In the presence of JA, 26S proteasome system along with the SCF^{COI} complex (containing the F-box protein CORONATINE-INSENTIVE1 (COI1), Cullin1 (CUL1), ASK1 (Apoptosis signal-regulating kinase 1)/ASK2(Apoptosis signal-regulating kinase 2, and Rbx1 (Ring-Box 1)) acts upon the JAZ proteins to degrade them, thereby releasing the genes *GL1* and *GL3* (Thines et al. 2007; Pattanaik et al. 2014). And, these genes form a complex with TRANSPARENT TESTA GLABRA 1 (TTG1) (Yoshida et al. 2009; Qi et al. 2011; Pattanaik et al. 2014) and positively regulate the expression of downstream targets, which ultimately leads to trichome induction (Qi et al. 2011; Pattanaik et al. 2014). Besides, JA has also been shown to specifically regulate the synthesis of type VI trichomes in some plants, besides mediating a wide range of other plant developmental processes. This has been confirmed by silencing the gene OPR3 (12-oxophytodienoate reductase 3), a precursor of JA, which largely undermined the density of type VI trichomes in tomato (Bosch et al. 2014; Huchelmann et al. 2017).

Using molecular genetics tools, studies have identified various transcription factors and genes controlling the development of various trichome types in different plants. For instance, in tomato, it has been suggested that the development of trichome type I is regulated by three genes – cyclin B2 gene, Woolly gene (Wo) responsible for encoding homeodomain leucine zipper (HD-ZIP) protein along with hair gene encoding zinc finger protein (Cys2-His2) that interacts with the Wo gene product (Marks and Feldmann 1989; Yang et al. 2011; Gao et al. 2017; Chang et al. 2018). Recent molecular studies on the hairless gene (*hl*) and its mutant from tomato clearly display its key importance in the trichome synthesis. This gene is responsible for the nucleation of actin, a protein responsible for the development of various trichome types (Kang et al. 2010, 2016). Also, the density of both glandular and nonglandular trichomes in tomato was found to be controlled by SlMIXTA1 with ectopic expression experiments, through an MYB transcription factor in tomato (Ewas et al. 2016, 2017). Additionally, it has also been suggested that the helix-loop-helix transcription factor, SlMYC1, is directly responsible for the synthesis of glandular trichomes in cultivated tomato plants – type VI trichome production consequently decreased with reduction in the levels of S1MYC1 and completely failed to develop in its absence/when turned off (Xu et al. 2018).

Role of Trichomes Against Abiotic Stresses

Drought, temperature extremities, intensive light intensities, salinity, heavy metal accumulation in the soil and water scarcity are the most common abiotic stressors on plants (Cakmak 2005). It is estimated that around 60% of soils pose growth restriction to the plants due to poor soil health and nutrition (Cakmak 2005; You and Chan 2015; Martínez-Natarén et al. 2018). These abiotic aggressors lower the plant yield between 50% and 80% (Martínez-Natarén et al. 2018) such that heat stress and drought coupled together led to $200 billion losses in the United States (Suzuki et al. 2014). However, various investigations on plant response to abiotic stress have also demonstrated the benefits of trichomes. Their effectiveness against abiotic stress largely relies on traits such as length, density, branching, and orientation, under the common umbrella “pubescence” (Hanley et al. 2007; Shanower 2008).

Trichomes have been found to physically defend the plants against water loss and heat stress (Gutschick 1999). Heat loss in pubescent leaves is accelerated through the process of convection leading to increased thermal conductivity of leaf surface (Dahlin et al. 1992). Consequently, plants undergo lower transpiration rates at higher trichome densities (Choinski and Wise 1999; Benz and Martin 2006; Burrows et al. 2013; Hauser 2014) and thus, regulate their water balance better (Hauser 2014). Clawson et al. (1986) showed that pubescent soybean varieties had 50% higher water use efficiency owing to the reduced evapotranspiration (Clawson et al. 1986; Dahlin et al. 1992). In addition, the presence of trichomes on the leaf surface also improves sunlight reflectance, therefore lowers the heat load on the plants, and consequently modulates and tolerates temperature variation and its impact on growth and development. Also, increased density of glandular trichomes (e.g., *Betula pendula*, family Betulaceae) was found to improve tolerance against frost by using trichomes as a structural adaptation under low-temperature regime (Prozherina et al. 2003). In addition, trichomes have also been implicated to play a role in the regulation of photosynthesis, by decreasing photoinhibition (Hauser 2014), and they can act as storehouses of UV-absorbing compounds like flavonoids (Hanley et al.,2007), thereby protecting the underlying photosynthetic tissues against harmful UV-A and UV-B radiations (Morales et al. 2002; Yan et al. 2012).

A large body of scientific studies have clearly documented that certain abiotic stresses like heavy metal contamination of soil and environment have been aggravated by anthropogenic activities (Straalen and Donker 1994; Yadav 2010; Wang et al. 2014; Wierzbicka et al. 2014). Rapid industrialization and current farming practices like heavy use of synthetic chemicals – pesticides and fertilizers – have been deteriorating the soil conditions to a great extent. Interestingly, trichomes can sequester heavy metals and assist in detoxification of the plants (Choi et al. 2001, 2004; Quinn et al. 2010). *Leontodon hispidus* (rough hawkbit, family Asteraceae), for instance, is known to accumulate calcium in its trichomes, whereas *N. tabacum*, on the other hand, has been shown to secrete zinc and cadmium through its trichomes (Glas et al. 2012), acquired from the soil. Besides, Mustafa et al. in 2017 and 2018 have proven the vitality of the biomineralization of the trichomes. They have suggested that mineralization of the trichomes majorly by calcium phosphate, calcium carbonate, and silica compounds calcifies them and thus, hardens them. This, in turn, strengthens their role as physical defenses by acting as stinging hair to the attacking herbivores (Mustafa et al. 2018). More interestingly, possessing trichomes is also a beneficial trait to the plant in outperforming competitors and thriving against unfavorable climatic conditions. For example, Han et al. (2019) showed that plants with trichomes, coupled with some other traits like upright leaves, a multilayered epidermis, or strong cuticles, have allowed them to outperform competitors in a forest succession (Han et al. 2019).

Collectively, trichomes have top-down effects as they effectively defend the plants against various abiotic stressors by reducing the heat load, lowering the evapotranspiration rates, and thus helping in maintaining water balance levels. Additionally, trichomes help protect photosynthetic tissues by absorbing damaging UV rays and accumulate harmful heavy metals.

Role of Trichomes Against Biotic Stresses

Besides abiotic stresses, plants also experience several and severe biotic stresses. These include insect pests, pathogens, weeds, and other animals which together cause about a 40% decline in crop productivity (Oerke and Dehne 2004). Among these, arthropod pests (mainly insects) cause ~ 15% crop losses per annum (Mitchell et al. 2016). Trichomes have been well studied in defending plants against biotic stressors. It has been found that nonglandular trichomes physically defend plants against herbivory. Like with the abiotic stresses, their pubescence plays an important role in defending against biotic aggressors – denser, prolonged, and more upright trichomes better defend against the insect attack (Shanower 2008). However, selective breeding for fitness traits can reduce the effectiveness of trichome-based plant defenses (Mitchell et al. 2016); consequently, cultivated species are found to possess lower density of trichomes as compared to their wild relatives (another line of reasoning on why cultivated crops are more prone to damage by insects than their wild relatives). For instance, 85% of neonate larval mortality in cotton bollworm (*Helicoverpa armigera*, family Noctuidae) was observed in wild pigeon pea (*Cajanus* spp., family Fabaceae) than the cultivated pigeon pea (*Cajanus cajan*, family Fabaceae). Interestingly, among other differences, wild pigeon pea pods possess twice the density of nonglandular trichomes when compared to the cultivated pigeon pea pods (Romeis et al. 1999). Higher density of trichomes successfully prevented *H. armigera* from reaching the pod surface, thus having them starve or desiccate to death, prior to their feeding initiation (Shanower 2008), as found on multiple *Solanum* species (Kariyat et al. 2018). Similarly, low infestation and sustenance of different caterpillars and beetles were observed in soybean varieties with denser trichomes than smooth-leaved soybean. In addition to protecting against chewing herbivores, it has also been shown that denser trichomes in wheat (*Triticum aestivum*, family Gramineae) restricted the movement of yellow sugarcane aphid (*Melanaphis sacchari*, family Aphididae) (Webster et al. 1994). Also, Chilean strawberry (*Fragaria chiloensis*, family Rosaceae) plants with dense trichomes were avoided by the black vine weevil (*Otiorhynchus sulcatu*s, family Curculionidae) (Doss et al. 1987), and cabbage white butterfly larvae (*Pieris rapae*, family Pieridae) inflicted greater damage on field mustard (*Brassica rapa*, family Brassicaceae) with less dense pubescence (Agren and Schemske 1993; Hanley et al. 2007). Szyndler et al., in 2013, showed a differed mode of trichome mediated defense. Nonglandular trichomes present on the leaf surface of the bean plants (*Phaseolus vulgaris*, family Fabaceae) was found to mechanically entrap bedbugs (*Cimex lectularius, family Cimicidae*) and kill them by the hooked sharp needle-like trichomes (Fig. 2.1c) – an interesting strategy for controlling bedbug infestations (Szyndler et al. 2013). However, adaptive features have been observed in the larvae of specialist herbivore *Heliconius charithonia* (family Nymphalidae) on the plant *Passiflora lobata* (family Passifloraceae) against the hooked trichomes borne on it. The insect was found to weave silken mats on the terminal of the hooked trichomes on the plant, speculated to facilitate the herbivore movement in their presence. Eventually, the presence of the hooked trichome tips in the insect feces suggested that they were successful in handling the mechanical defenses of the plant, while generalist insects (*Heliconius pachinus*, family Nymphalidae) were found struggling with the defenses and had a higher mortality rate (Cardoso 2008).

Additionally, nonglandular trichomes have been found to possess a varied mode of action against insects at different life stages. Plant surface bearing trichomes is an unfriendly landscape for majorly soft-bodied chewing insects like the lepidopteran larvae. This is more evident in the case of neonate caterpillars, which tend to feed on the leaf epidermis upon emergence. As previously shown by Kariyat et al. in 2017 and 2018, trichome-bearing *Solanum* leaves pose difficulties to the first instar larvae of *M. sexta* to initiate feeding. They struggle to reach the leaf epidermis in the presence of spine-like stellate trichomes which causes them to starve, desiccate, and ultimately die. However, both glandular and nonglandular trichomes are ineffective against the late instar larvae in disrupting their feeding. Interestingly, stellate trichomes (nonglandular) were found to poke through the early instar larvae, rupturing their tender epidermis to leak hemolymph and ultimately leading them to death.

Glandular trichomes are more commonly found to produce defense chemicals in plants. These may constitute up to 30% of the plant's dry leaf weight. Depending on the plant family and species, the glandular trichomes have been found to possess a diverse range of biologically active defense compounds such as alkaloids, glucosinolates, cyanogenic glycosides, terpenoids besides latex, and protein inhibitors (Glas et al. 2012; Tissier 2012; Kariyat et al. 2018). These compounds affect herbivory by affecting insect metabolism, membrane disruption, inhibition of signal transduction processes, nutrient and ion transport, or other physiological processes, collectively leading to reduced feeding, growth, development, and even mortality in the affected herbivore species (Shanower 2008; Glas et al. 2012; Kariyat et al. 2019). Besides, these glandular trichomes are also reservoirs for terpenes, aromatic compounds, and fatty acid derivatives. These compounds, which are predominantly volatile (volatile organic compounds (VOCs)), defend the plants indirectly by the attraction of natural enemies of the herbivores feeding the host plant (Weinhold and Baldwin 2011).

Besides, studies have been conducted on the post-feeding impacts of trichomes on insect behavior and physiology. They have been found to interfere with the insect digestive system (Shanower 2008). Consumption of trichomes is a commonly observed behavior in chewing caterpillars. To gain access to the underlying leaf tissue for infestation/feeding, caterpillars tend to feed on the trichomes, eventually consuming them. Kariyat et al. (2017) showed that consumption of nonglandular trichomes isolated from the weed Carolina horsenettle (*S. carolinense* L., family Solanaceae) led to breaching of the peritrophic matrix – the gut membrane, which plays a key role in the defense, in tobacco hornworm (*M. sexta*, family Sphingidae) larvae post-ingestion. This is a vital study that implies trichomes to be major plant defenses by themselves, without any coupled effects of other structural and nonstructural defenses (Howe and Jander 2008; Kariyat et al. 2013). Manipulative studies decoupling trichomes from other defenses have shown that trichomes negatively impact both the growth and development of caterpillars (Kariyat et al. 2019). Therefore, trichomes can resist herbivory both before and after the insect feeding is initiated – leading to pre- and post-ingestive effects (Kariyat et al. 2017).

In addition, genetic variation has been found to play an important role in trichome-mediated defenses against herbivory. Kariyat et al. (2013) demonstrated that both abaxial and adaxial trichomes are found to significantly vary among maternal families in Carolina horsenettle, and even more importantly inbreeding affected both constitutive and induced trichome productions. A recent study from the same group (Nihranz et al. 2019) showed that not only does inbreeding lower the plant defenses by reducing the trichome production (Kariyat et al. 2019; Kariyat and Stephenson 2019) in *S. carolinense* upon damage by *M. sexta* but also compromise their induction over the generations. Not surprisingly, they also reinforced the fact that higher trichome density post damage (induced) was found on plants under herbivory as compared to the undamaged control plants.

Hence, the consensus is that higher trichome density positively correlates with resistance to herbivory (Glas et al. 2012). This could be achieved by restricting herbivore access to the epidermis (Kariyat et al. 2018), thereby delaying feeding (Kariyat et al. 2017), or impeding growth (Kariyat et al. 2018) and development (Kariyat et al. 2019) upon consumption of trichomes. And, while nonglandular trichomes are more effective as a structural defense (Lanning and Eleuterius 1985), it is also clear that they are more effective against neonates and early instars, as seen in the plant family Solanaceae (Kariyat et al. 2018). Since the first cue for imminent herbivory on plants is oviposition, studies have also examined whether trichomes can affect oviposition. For example, the herbivore Chinese bruchid (*Callosobruchus chinensis*, family Chrysomelidae) prefers oviposition on glabrous pigeon pea pods over the hairy type, and cereal leaf beetle (*Oulema melanopus*, family Chrysomelidae) prefers to oviposit on wheat cultivars with lower trichome density. On the contrary, oviposition and trichome density have been found to positively correlate in soybean and cotton cultivars. For instance, *Helicoverpa* spp. (family Noctuidae), Western tarnished plant bug (*Lygus hesperus*, family Miridae), spiny bollworm (*Earias vitella,* family Noctuidae), and cotton-spotted bollworm (*E. fabia*, family Noctuidae) lay more eggs on cotton with higher trichome density. Similarly, bean fly (*Ophiomyia phaseoli*, family Agromyzidae) and soybean pod borer (*Laspeyresia glycinivorella*, family Olethreutidae) prefer soybean pods with higher trichome density than smooth pods (Shanower 2008). While there are contrasting examples, in general, both oviposition and neonate larvae survival are found lower on plants with more trichomes (Kariyat et al. 2017, 2018). It is plausible to speculate that the presence of trichomes and any associated cues (tactile or volatile) might be used by the ovipositing females as an honest signal for host location, in these few cases – an area that must be explored in detail.

Besides density, other factors of pubescence such as length and morphology of trichomes can effectively restrict herbivory, especially by the small-bodied insects. For instance, prolonged glandular trichomes were found to successfully defend the leaves of the plant field beans (*P. vulgaris*), against potato leafhopper (*Empoasca fabae*, family Cicadellidae), irrespective of trichome density (Shanower 2008). Potato leafhopper is a small-bodied insect pest mainly responsible for causing devastating hopper burn symptoms in crops. And, pink bollworm larvae (*Pectinophora gossypiella*, family Gelechiidae) on cotton cultivars were eventually found dead of starvation and desiccation after their failed struggle to move around on the leaves of the plant to initiate feeding (Shanower 2008).

The efficiency of metabolite allocation and energy administered to the defensive activities in plants is based on the adaptive modulation of plant metabolism, recognition of the herbivore, and precision in host plant's activity with intercellular signaling and accurate biochemical, physiological, and cellular responses (Maffei et al. 2007a, b; Mithöfer and Boland 2012). And, due to the dynamic nature of herbivore infestation, and host-herbivore species specificity (e.g., specialists vs generalists), studies have also found that the structure, density, and distribution of trichomes vary at both inter- and intraspecific levels, with implications for trade-offs in the plants for resource allocation toward nutrition and/or defenses (Kariyat et al. 2013; Hauser 2014).

Trichomes and Their Role in Direct and Indirect Defenses

As a part of the herbivore-resistance mechanism, glandular trichomes release specialized metabolites that are directly toxic, feeding deterrents, or discouraging insect oviposition. Their appendages mediate the toxin delivery to the herbivores that discourages their feeding activity (Agren and Schemske 1993; Agrawal et al. 2004; Kariyat et al. 2019). Since trichomes are the storehouses for various toxins, it gives rise to the potential risk of self-intoxication. To prevent this, trichomes usually store these phytochemicals in their vacuoles or apoplasm, thereby minimizing the risk (Mithöfer and Boland 2012). Upon the attack by a herbivore, the glandular trichomes rupture, causing the toxic exudates to be released to the plant surface. Upon oxidation of released compounds (e.g., polyphenols and O-acyl sugars), the exudates become sticky leading to the entrapment of the arthropods on the surface. Ultimately, the insects die of starvation or toxicity due to consumed plant metabolites or suffocation, or they activate jasmonic acid-mediated defense gene expression, leading to both site and systemic defenses (Peiffer et al. 2009; Tian et al. 2013).

In addition, the toxic metabolites produced by the glandular trichomes also aid in systemic immunization of the attacked plant. These defense compounds are found to be transported to distal plant parts, thereby priming the plant against herbivory. Pyrethrins, found in *Tanacetum cinerariifolium* (family Asteraceae) are produced in the glandular trichomes present on the fruits. Eventually, the pyrethrins from the fruits are transmitted to the seedlings via the seeds. Astonishingly, these seedlings which are trichome-free themselves are found to be insect- as well as pathogen-resistant/pathogen-tolerant (Glas et al. 2012; Mithöfer and Boland 2012). Besides, various studies have shown anti-herbivore activity of sesquiterpene lactones found in the trichome extracts of *Tanacetum* spp. For instance, *Spodoptera littoralis* (Mediterranean brocade, family Noctuidae) feeds poorly on *T. cadmium* ssp. *cadmium* and *T. corymbosum* ssp. *cinereum* flowers as they have sesquiterpene lactones with antifeedant activity (Gören et al. 1994).

To defend indirectly, glandular trichomes have been found to release volatile organic compounds (VOCs) leading to the attraction of natural enemies of the infesting insect pest species (De Moraes et al. 1998; Walling 2000; Heil et al. 2001; Mithöfer and Boland 2012). VOCs vary in their composition (Mithöfer and Boland 2012) and mostly comprise of terpenes, phenylpropanoids, glycerides, etc. (Kariyat et al. 2012a, b; Tissier et al. 2017). Their composition largely varies with the type of insect, feeding damage, mode of damage, insect oviposition or host plant genetics (Kariyat et al. 2012a; Mithöfer and Boland 2012). Predatory insects are attracted by the phytochemicals produced by the glandular trichomes. For example, O-acyl sugars produced by the glandular trichomes of *N. tabacum*, upon consumption by the neonate caterpillars of *M. sexta*, render them attractive to the predatory ants (Weinhold and Baldwin 2011). On the contrary, few studies have also shown that trichomes can aid herbivores to escape predation or parasitization by their natural enemies. In the presence of trichomes, natural enemies usually have to spend more time searching for their prey. Additionally, they are more prone to physical entrapment in the toxic sticky exudates and are affected by chemical repellants emitted from the secondary metabolites of trichomes. For instance, *Trichogramma* spp. (egg parasitoid) (family Trichogrammatidae) have been found to have faster locomotion in search of prey on the glabrous leaves than the trichome-bearing leaves of cotton and pods of pigeon pea (Shanower 2008). In another study, locomotion of the predatory green lacewing (*Chrysoperla carnea,* family Chrysopidae) was found to be hampered on trichome-bearing plant California pipevine (*Aristolochia californica*, family Aristolochiaceae) eventually protecting the larvae of pipevine swallowtail (*Battus philenor*, family Papilionidae) against predation (Fordyce and Agrawal 2001). However, the feeding damage by the caterpillars was restricted to less than 30% on the same plant, owing to the trichomes (Mithöfer and Boland 2012).

Although a significant amount of research has been conducted to examine the chemical composition of trichomes, a common bottleneck is the lack of easy and precise methodology to isolate the trichomes without contamination from extraneous plant matter. Various traditional isolation techniques involved methods like shaving the trichomes off the leaf blade with a scalpel blade (Croteau 1977; Kariyat et al. 2017), abrasion of the leaf surface with a microscope coverslip (Keene and Wagner 1985; Yerger et al. 1992), a glass slide (Keene and Wagner 1985; Tissier 2012), and a brush (Yerger et al. 1992) and the use of liquid nitrogen-based extraction (Kariyat et al. 2019) to name a few. The leaf samples are usually placed in a buffer before brushing the trichomes off the surface. Additionally, some studies also use an adhesive tape (Piazza et al. 2018) or double-sided tape attached to a glass slide (Gopfert et al. 2006; Tissier 2012) to isolate the trichomes. This method involves detaching the trichomes from the sticky surface either by submerging the tape in water or by placing it in different kinds of buffer solutions and vortexing the container or removal of trichomes using a paint brush. Then, the trichomes are filtered by passing the solvent-containing trichomes through a fine metal meshes sized 100 μm and 80 μm, consecutively (Piazza et al. 2018). Methods using swabbing the leaf surface with cotton submerged in antioxidant (Kowalski et al. 1990), using Percoll density gradient centrifugation (Yerger et al. 1992; Tissier 2012), using glass beads and mesh technique for cryopreserved plant tissues (Ranger et al. 2004; Tissier 2012), and abrading the leaf surface with dry ice (Yerger et al. 1992; Tissier 2012) have also been used with different levels of success. Recently, it has been shown that a new technique using pressurized cyclic solid-liquid extraction using Naviglio Extractor, is about 15 times more efficient than the traditional methodologies in harvesting the trichomes, as found in *Artemisia annua*. This technique involves compressions and immediate decompressions on the plant tissue, which subsequently creates a pressure gradient that ultimately leads to the extraction of trichomes without any actual maceration (Zarrelli et al. 2019).

Future Directions

As outlined above, significant research has been conducted to understand the role of trichomes in plant-herbivore interactions, in both nondomesticated and domesticated species. However, we still lack a comprehensive understanding of certain aspects of trichomes.

The majority of the studies conducted in the past have mainly focused on trichomes in the cultivated crops or their wild relatives (Glas et al. 2012; Livingston et al. 2019). However, few have targeted flora related to forest ecosystems and horticultural fruits and vegetable crops (Li et al. 2015; Liu et al. 2016, Wang et al. 2016, Luján et al. 2018). Therefore, we need to study and characterize the trichomes in these systems as well. While various studies have described the role of trichomes against different abiotic stressors to the plants, we lack the understanding of how key abiotic factors influence the trichome development and induction and their effects in growth-defense trade-offs (Kariyat et al. 2013).

Recent developments in the use of molecular genetics in trichome research have led to the creation of cDNA libraries from various plants – *Solanum* sp., *M. piperita*, *O. basilicum*, etc. However, more elaborative studies aiming at the expression and quantification of genes associated with trichome biosynthesis and biochemistry in different plants are required – with a possible target of genetically engineered plants with efficient defense allocation in trichomes without compromising the yield. Also, such studies can be used to understand and regulate the mechanisms responsible for the synthesis of secondary metabolites and their transport throughout the plant organs.

We also speculate that nonglandular trichomes can possibly do more harm than understood so far. Since these trichomes physically breach the young larvae (pierce through their skin), they inflict mechanical injury to them (Kariyat et al. 2017, 2018, 2019). Besides, it can be speculated that microbes harbored on the trichome surface are also potentially injected and translocated during the process. Hence, the aftereffects of these microorganisms post their transfer into the insect body need a detailed investigation – role in mediating multi-trophic interactions, effects on insect metabolism, etc. Few studies have addressed these questions so far (Lahlali and Hijri 2010; Kim 2019). And, finally, we need to assess convenient and efficient methodologies/protocols to extract intact trichomes, without harming the leaf epidermis. This would expand horizons for conducting more elaborative and comprehensive research in various aspects like histochemical and ultrastructural microscopy-based studies.

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Chapter 3
Resource Allocation and Defence Against Herbivores in Wild and Model Plants

Germán Avila-Sakar

Abstract Tolerance of herbivory has been defined as the ability of plants to maintain fitness despite being damaged by herbivores. It is now recognized as a complementary mode of defence to resistance – the ability of plants to avoid being damaged by herbivores – but for a long time, any indications of equal or greater fitness, growth or other measures of performance of damaged plants compared to undamaged ones were dismissed as the results of methodological flaws. In this chapter, I present an account of my own immersion into this field, and my view of the dire need to understand thoroughly the physiological mechanisms of plant defence against herbivores (mostly still lacking for tolerance) so that we can fully understand the ecology and evolution of plant-herbivore interactions. I underscore the central role of resource allocation theory in our understanding of plant defence and the importance of modelling to link theoretical and empirical approaches to the study of tolerance of herbivory. I highlight the contributions of my research programme using *Arabidopsis* to address predictions on the ontogenetic trajectories of tolerance and resistance to herbivores. My interest in the possible role of herbivory in the evolution of dioecy brought me to study a wild population of *Ilex glabra*, where I could test hypotheses of sexual dimorphism in defence. I devote brief sections to the difficulties and remaining questions in the study of tolerance, the costs and benefits of resistance and tolerance and the factors that affect them. The discovery that endoreduplication is associated to overcompensation is, perhaps, the most promising step so far in our elucidation of the mechanisms of tolerance to herbivory, and perhaps it signals the time to start conducting more studies in wild populations so as to test the generality of the knowledge we have gained from the study of model species.

Keywords *Arabidopsis* · Costs · Dynamic model · Endoreduplication · Ontogenetic trajectories · Overcompensation · Principle of allocation · Resistance · Tolerance · Trade-offs

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When I started working on my main project as a doctoral student, I was not very enthused because I thought it was mostly ‘confirmatory’ rather than ‘ground-breaking’. For reasons that at the time seemed convoluted, I was studying the effects of the spatial pattern of herbivore damage on the male and female components of fitness of a weedy squash. My mood worsened as I started analysing the data from my first field season, and the results looked nothing like what my supervisor and I expected based on the literature on the effects of herbivory on plant fitness (e.g. Crawley 1983). Several of the variables I was using as indicators of plant fitness were not changing significantly in response to damage. Moreover, one of them, pollen siring ability, improved under damage (Avila-Sakar et al. 2003). The onus was on me to show that I had not messed up the simulated herbivore damage treatments and that plants were actually responding the way my data showed. I had to admit I had no idea why plants were doing what they were doing, or how it was possible.

In the coming months and years, I embarked on the enterprise of making sure I applied the damage treatments ‘correctly’ on the plants, which meant, I needed to understand better what a particular spatial pattern of damage meant in terms of how the damage affected the physiology of a plant. In doing this, I switched my experimental units from individual leaves to branches, branch systems and ultimately, whole plants (Avila-Sakar et al. 2003; Avila-Sakar and Stephenson 2006). It was then that I started realizing ever more clearly that hypotheses generated under thorough evolutionary and ecological theory required also to be grounded on a physiological context. In other words, whatever we predicted that plants should be doing in response to damage had to be possible based on the physiological mechanisms governing the responses of plants to damage.

The present chapter was thought as a guide through some of the conceptual changes, challenges and advances in our understanding of plant defence against herbivores with particular emphasis on tolerance to herbivory (the degree to which plants can maintain fitness despite having suffered damage by herbivores), all within the framework of resource allocation theory. First, I examine the relation between expectations of plant responses to herbivores derived from resource allocation theory and the physiological bases of those responses. I draw from my research programme to present examples of studies that addressed some of the questions that arose as those of us in this field realized the prevalence of tolerance as one of the modes of plant defence against herbivores (Karban and Baldwin 1997). I examine some of the factors that alter tolerance to herbivory and stress the need to understand the mechanisms of tolerance at least with the level of detail with which we now understand the mechanisms of resistance (the ability of plants to decrease damage by herbivores). I also include a brief analysis of the recently discovered role of endoreduplication in tolerance to herbivory. Finally, I present some ideas on the costs and benefits of allocation to resistance and tolerance traits and some of the factors that may alter these costs.

Theoretical Costs and Benefits of Resource Allocation to Defence

In theory (I thought), plants that lost photosynthetic tissue to herbivores should suffer reductions in fitness as measured by the number of seeds produced (female function) or the number of seeds sired on other plants (male function). The main reason to expect such a decrease in fitness is that the production of reproductive structures (directly contributing to fitness) is determined strongly by the amount and functionality of vegetative tissues that serve both to acquire the resources needed to construct reproductive structures and to bear physically those structures (Harper 1977). Also in theory, the allocation of resources to one function (maintenance, growth, defence and reproduction) reduces the amount of resources available for other functions (Cody 1966; Harper 1977). Consequently, a plant that allocates resources to defence mechanisms induced in response to herbivore attack will also have a reduced amount of resources left to produce reproductive structures. This apparent double whammy to a plant's ability to acquire resources (transformable into fitness) makes it difficult to explain the absence of negative effects of herbivory on fitness, let alone any positive effects. In addition, from an evolutionary perspective, one would have to explain why a genotype that achieves greater fitness with less leaf area has not become widespread in a population as a result of natural selection.

Clearly, the task of convincing my supervisor at the time, and the reviewers of my manuscripts in the years to come, that plants damaged by herbivores could compensate (achieve equal fitness to that of undamaged plants) or overcompensate (achieve greater fitness than undamaged plants) was not an easy one. Something was wrong with our logic if plants were doing things we did not expect them to do. To figure out what was wrong, I needed to understand better how it was that plants did what they did in response to herbivore damage. In other words, I needed to understand plant physiology much better than I did.

First, let us consider the expectation that a decrease in photosynthetic tissue should result in decreased fitness. Photosynthesis is the process through which plants gain C from CO_2 in the air (carbon fixation), and in most plants, it occurs primarily in the leaf mesophyll. Thus, it should follow that any loss of leaf tissue (photosynthetic) should result in a decrease in the amount of C fixed. However, the rate at which C is fixed is not constant, as it is regulated through several feedback mechanisms: it slows down when sucrose (the main transport sugar) accumulates in the mesophyll cells (the main sources of photosynthates) thus decreasing the demand of triose phosphate (end-product of the Calvin cycle), which in turn decreases the recycling of the Pi needed for photophosphorylation (Paul and Foyer 2001). Sucrose is transported via the phloem from mesophyll cells to places where it is needed as a source of energy or C atoms for structural carbohydrates (sinks). The loss of leaf tissue triggers repair mechanisms that involve the use of energy and structural molecules (including carbohydrates), thus raising the demand for C (and other elements). By depleting the sucrose available in mesophyll cells, these temporary sinks cause increases in photosynthetic rates that can last from hours to days, purportedly allowing the photosynthetic rate to reach its maximum for a given photon flux density. Photosynthetic rate increase in response to insect feeding and ungulate browsing has been found in a number of species, including *Ilex aquifolium* (Retuerto et al. 2004), *Eucalyptus globulus* (Pinkard et al. 2011) and *Populus tremuloides* (Rhodes et al. 2017), but are not necessarily a universal plant response (Tiffin 2000 and refs. therein). However, herbivory induces the downregulation of photosynthesis-related genes, which can suppress photosynthesis on damaged leaves (Nabity et al. 2009). The details of the feedback mechanisms may seem overwhelming at this point, but they are part of what evolutionary ecologists have missed when making predictions about fitness in relation to available leaf area.

Leaves are also the primary organs for transpiration, the loss of water by evaporation through stomata, a process that generates the negative pressure that drives the movement of water from the soil to the roots, stems and leaves (Canny 1990). This movement of water through the plant, the transpiration stream, is necessary for the absorption of mineral nutrients through the roots. As with photosynthetic rate, the transpiration rate is not constant. It depends on the number of stomata that are open at a given time and on how big and open they are (Dow et al. 2014). Stomatal conductance (the rate of passage of gases – viz. CO_2 and water vapour – through the stomata) is regulated by water availability in conjunction with abscisic acid. Normally, the epidermal layer is highly impervious to water. Thus, upon its breakage by chewing herbivores (or abiotic factors), water loss occurs through the exposed leaf tissue layers at the wound site until these tissues (especially the xylem) are functionally severed, and thus isolated, from the surrounding healthy leaf tissues. The loss of water brought about by damage should trigger the closure of stomata (Nabity et al. 2009 and refs. therein). In summary, leaf damage should first result in a sudden increase in evapotranspiration followed by a decrease to evapotranspiration levels lower than normal or even those characteristic of plants in drought stress (Peschiutta et al. 2016). The effects of such changes in evapotranspiration on nutrient absorption through the roots will depend on the extent and duration of damage and the duration of the resulting phases of increased and decreased evapotranspiration.

Thus, following wounding, a leaf will experience increased photosynthetic rates and transpiration, which should bring in more C and mineral nutrients than an undamaged leaf per unit area and time. Interestingly, a greenhouse study on six legume species showed no changes in photosynthetic rate in response to clipping or actual insect damage (Peterson et al. 2004). Since one of the most limiting nutrients is N, I will focus on N for the remainder of this section. Whether the net gains are greater than those of an undamaged leaf will depend on the area of the remaining leaf tissue and the total C and N gained during the period of increased photosynthetic and transpiration rates compared to those of an undamaged leaf on an undamaged plant (Harper 1989). Moreover, cellular respiration may also increase following damage due to the triggering of biochemical pathways leading to apoptosis of nearby cells to contain the flow of water out of a wound, but increases and decreases have been reported (Pinkard et al. 2011 and refs. therein). If respiration increases with damage, a greater expenditure of C would be expected near the wound sites. Moreover, due to the activation (possibly also involving respiratory costs of signalling) of dormant meristems or the mobilization of stored resources, one would expect the activation of biochemical pathways that would establish priority of those new sink tissues and processes over other processes associated with the normal growth and developmental programme of an undamaged plant.

In summary, it is not so straightforward to expect a decrease in total C and N gains for any given decrease in leaf area due to herbivory, whether at the individual leaf level or at the whole plant level (whereby some leaves have been wounded, but others have not). Still, to propose physiologically sound hypotheses about the evolution and ecology of plant-herbivore interactions, we need a thorough understanding of the molecular and cellular mechanisms that determine the allocation priorities of plants that could be encapsulated in two questions: (1) How much lifetime total C and N is available to a plant that has been wounded once? (2) How do those quantities compare with the ones for an undamaged plant?

Assuming we get an answer to the first question, the next step is to consider the fate of the total C and N gained by a plant that has experienced damage and how those resources are converted into fitness. We need to consider the whole plant because fitness is defined at the individual level. We need to know whether the fates of C and N change in response to wounding and for how long. In other words, do the allocation priorities of C and N change with wounding, how and for how long? Do shoot apical meristems get more C and N? Does this draw resources away from root apical meristems? Does this depend on whether the plant is at the vegetative or reproductive stage? These questions can be approached from the perspective of sink-source relationships (Farrar 1993, 1996).

It is important to emphasize that fitness depends directly on the number and effectiveness of reproductive structures, which number, size and efficiency at producing progeny depend on the existing vegetative infrastructure. Reproductive structures can be seen primarily as resource sinks, but they can also contribute importantly to their own resource expenditures (Bazzaz et al. 1979; Earley et al. 2009). To complicate things further, the changes in allocation priorities may depend on the type of tissue damaged (e.g. foliar vs. meristem).

Perhaps the most evident change in allocation priorities is the added resource expenditure on the defence-related signalling pathways and the consequent synthesis of volatiles and secondary metabolites. It is tempting to jump into the conclusion that these added expenditures *must* decrease the availability of resources. However, the generation of new sinks can stimulate resource acquisition, as we saw above. Thus, the feedback mechanisms that regulate carbon fixation and the transport electron chain actually prevent these biochemical-cellular systems from functioning continuously at maximum rates (Pinkard et al. 2011). Without the feedback mechanisms, much waste and cellular damage would occur, and certainly homeostasis would never be achieved.

Detailed answers to the questions posed above will allow for more realistic predictions about the costs and benefits of defence and the adaptive value of allocation to defence. This detailed knowledge will help us transition from the simpler thinking based on a static, single-damage/response view to a perspective that considers dynamic changes in resource acquisition and use throughout the lifetime of plants.

My Contributions to Answering Some of These Questions

From Models to Experiments

My research programme has contributed towards answering these questions through a combination of empirical and theoretical approaches that addressed various levels of detail of the physiological mechanisms governing plant responses to damage in terms of total resource acquisition and allocation priorities. On the more theoretical side, together with a masters student of mathematics, I expanded on the physiologically based Growth Rate Model (Hilbert et al. 1981) to examine the common assumption that plant fitness decreases in a simple linear fashion as the amount of tissue damage increases (Avila-Sakar and Laarakker 2011). While this assumption is appealing for its simplicity, it is not realistic for many of the reasons listed in the previous section. The possibility of a convex tolerance function is highly relevant to the studies of the evolutionary ecology of plant-herbivore interactions because it modifies the expectation that damage should invariably cause a decrease in fitness and opens the possibility that damage could have positive or null effects on plant fitness depending on the exact shape of the function for a particular plant-herbivore system. Our model (hereafter, the Expanded Growth Rate Model or EGRM) showed that the tolerance function is more likely to be curvilinear because of the way in which the relative growth rate changes in response to damage (Avila-Sakar and Laarakker 2011). The curvilinearity allowed for positive effects of damage on fitness (overcompensation), perhaps being the first theoretical derivation of a response found not so uncommonly in empirical studies, including some from my doctoral project (Avila-Sakar et al. 2003; Avila-Sakar and Stephenson 2006). A convex tolerance function would favour the evolutionary stability of a mixed defence system, characterized by simultaneous allocation of resources to resistance and tolerance traits, thus supporting that mixed defence systems should be more common than those mostly based on resistance or tolerance (Núñez-Farfán et al. 2007). In addition, and contrary to the more intuitive notion that faster-growing plants would be more tolerant to damage (Agrawal et al. 1999; Coley et al. 1985), our EGRM predicted greater tolerance of plants with lower growth rates. I have found empirical support for this prediction in *Arabidopsis* (Tucker and Avila-Sakar 2010). Much earlier, a study on three different clones of the grass *Themeda triandra* found an association between a lower relative growth rate and greater compensatory ability (tolerance). Similarly, experiments on *Datura stramonium* (Solanaceae) support that plants with intrinsic lower relative growth rates are more tolerant to herbivory (Camargo et al. 2015).

While the GRM model is based on the knowledge of growth rates in plants, derived, in turn from growth analysis experiments, it lacks details of the physiological mechanisms involved in achieving a particular growth rate (Hilbert et al. 1981). Still, it is one of the first papers to call attention to the physiological mechanisms that allow for compensatory growth in plants. Interestingly, in their paper, Hilbert et al. expressed doubts that these would be sufficient to compensate fully for ‘decreased production following damage’.

In a different approach to understanding the physiological mechanisms involved both in resource acquisition and the establishment and modification of allocation, Reekie and Avila-Sakar (2005) examined the cost of reproduction: How much does allocation to reproduction limit vegetative growth? It is not difficult to imagine that, if the production of reproductive structures depends on a vegetative infrastructure, excessive or untimely (too early) allocation of resources to reproductive structures could ultimately incur a fitness cost by limiting the production of vegetative structures that would eventually support the production of more reproductive structures, effectively decreasing the number of reproductive structures produced over an individual’s lifetime. We were particularly interested in the shape of the cost function: simple-linear or curvilinear. A simple-linear function means that an increase in current reproduction results in a proportional decrease in future reproduction. The cost function could be curvilinear for a variety of reasons, including shared costs of construction, contribution of reproductive tissue to photosynthesis and differences in resource requirements between vegetative and reproductive tissue (Lord and Westoby 2006; Reekie and Avila-Sakar 2005; Reekie et al. 2002).

An empirical study of the effects of resource availability on the cost function would require the experimental manipulation of both the availability of resources in the environment and the resource requirements of vegetative and reproductive structures. As explained by Reekie and Avila-Sakar (2005), while resource availability can be experimentally altered without much difficulty, manipulating the resource demands of vegetative and reproductive tissues without major unwanted effects could require very precise and localized alterations of gene expression, not without possible pleiotropic effects. To circumvent this difficulty and other limitations of empirical studies, we used an 18-parameter computational model that, while still simplified, used as much detail of physiological processes to simulate the growth of a plant and its allocation ‘decisions’ occurring almost instantaneously – a dynamic model sensu Thornley (1998). Our model simulations always produced convex cost curves because the relationship between reproductive output and reproductive investment always has a maximum (i.e. it is a hump-shaped curve): the decline in fitness became steeper as the proportion of resources allocated to reproduction increased. This is due to opportunity costs incurred by not investing in the vegetative infrastructure that ultimately would sustain more reproductive structures which in turn procure fitness for an individual. We also showed that the cost of reproduction decreased under conditions that allowed high growth rate (e.g. high carbon and nitrogen availability), and it practically disappeared at low values of reproductive investment (Reekie and Avila-Sakar 2005). This model allowed us to lay out several predictions regarding the influence of resource availability on tolerance that we addressed in an experiment with *Brassica rapa* (Marshall et al. 2008).

Our model was teleonomic: we decided how much a plant allocated to reproduction by setting the amount of C and N that would be devoted to reproduction as a fixed proportion of the C and N acquired by the plant. It is unlikely that plants have such a rigid developmental programme. The actual mechanisms that determine C and N allocation to reproduction most probably include a mixture of canalized and plastic traits. For instance, a particular pattern of meristem production (canalized) may determine the number of flower buds initiated per inflorescence. However, as buds develop, the concentration of different phytohormones (elicited by various exogenous and endogenous conditions) may alter the number of flower buds that complete their development (plastic). During the time of flower bud development, the demand for C and N from the buds (sinks) may elicit the shunting of C and N to the flower buds, and simultaneously influence the rates of acquisition of C and N at the source organs. Thus, resource availability for a particular organ or function will be influenced both externally by ambient availability and internally by the demands from other organs in the plant (sinks) (Marcelis 1996).

Despite simplifications made at the cost of certain degree of realism, the model allowed us to see how an exaggerated allocation to reproduction results in lower fitness (as measured by reproductive biomass) than a lower reproductive investment. The reason for this is that the allocation of resources to reproductive structures takes resources away from growth, ultimately resulting in a reduced capacity to produce reproductive structures, and in turn, lower fitness than a lower allocation to reproduction – something that initially could seem counterintuitive. Conversely, too little reproductive investment will also result in lower fitness than some intermediate amount of reproductive investment. This computational model could be modified to account for loss of leaf, stem or root mass to herbivores to assess whether the predictions of the EGRM are met. In particular, it could be used to check whether overcompensation occurs under conditions that make plants grow slowly.

One important contribution of the dynamic model is that it forces us to think of resource availability also as a dynamic amount. Available resources are not a fixed pool, often represented by a circle that conveys the idea of a static amount of resources in the environment from which a plant can draw and have its own fixed pool of acquired resources. Instead, we must think of resource availability as a balloon that changes in volume as resource demand and acquisition capacity change (dictated by the number and efficiency of organs involved in acquiring the different resources). Our model highlights the dynamic nature of resource availability by explicitly including the external resources and an internal pool of resources as parameters that can be changed (Reekie and Avila-Sakar 2005).

Maynard Smith (1968) pointed out that mathematical models are useful to understand what needs to be measured. In this case, the models we developed helped me get a better idea of what we needed to know in order to understand what determines allocation priorities in plants, even without a thorough understanding of the underlying physiological mechanisms: lack of nutrients would signal a priority to allocate resources to roots, while lack of carbon compounds would signal a priority to the growth of the shoot, including the elongation of the stem and the production of more leaves. Interestingly, similar ideas were being developed in what became known as the limiting resource model (LRM) to explain the conditions under which plants would compensate depending on whether the damage caused by herbivores affected the uptake of a resource that was already limiting or not (Wise and Abrahamson 2005).

We conducted an experiment with *Brassica* to study the effects of resource availability on the tolerance of herbivory (Marshall et al. 2008). We were able to test the predictions of three models: the compensatory continuum hypothesis (CCH) (Maschinski and Whitham 1989), the GRM (we had not yet published the EGRM) and the LRM by varying the availability of two resources, CO_2 and nutrients (mostly N, P and K) in a crossed factorial design of high and low availability of CO_2 and nutrients. In short, the GRM predicted that plants with low growth rates would have greater tolerance than those with high growth rates. The CCH predicted exactly the opposite, arguing that plants require more resources to recover from the loss of tissue to herbivores, but without an underlying physiological framework. The LRM proposes that the degree to which plants can compensate for herbivore damage depends on whether it affects the uptake of a resource that is limiting growth or not.

Our results supported neither model, and rather suggested that both resource uptake and allocation are plastic, but in a more complicated way than the tested models propose (Marshall et al. 2008). In fact, our plants were generally tolerant to 15% leaf area loss to *Trichoplusia ni* larvae: by the end of the reproductive period, plants had equally compensated under all conditions of resources. However, plants growing under low availability of both nutrients and carbon grew at a lower rate than those of other treatments, and thus, should have been the only ones with a different response according to GRM or CCH. The LRM predicted that plants growing under high carbon availability should have had an enhanced compensatory response (higher tolerance) because of the putative (and questionable) negative effect on carbon uptake of the removal of leaf tissue by herbivores. Simultaneously, the LRM predicted a diminished compensatory response (lower tolerance) under high nutrient availability when herbivory decreases the uptake of an alternate resource (i.e. carbon). We did not see either of these outcomes in our experiment.

This particular experiment shows that while our predictions were solidly based on models that attempt to consider the physiological basis of plant responses, we still lack more detailed knowledge of the effects of resource availability on growth rate, leaf production and senescence, onset and duration of the reproductive stage and rate of fruit maturation, let alone the responses to herbivores.

Resource Allocation and Herbivory in Dioecious Species

The evolution of unisexuality and herbivory in dimorphic species (mainly dioecious and gynodioecious) poses a similar set of problems regarding our expectations of the evolution of new patterns of resource allocation resulting from changes to the sexual system and the actual genetic, molecular, cellular and physiological mechanisms that would allow plants to establish the expected allocation patterns. There is a pronounced asymmetry between the level of detail and care with which the theory of sex allocation has been developed (Barrett 2002; Charlesworth 1999; Charnov 1982; Lloyd 1984) and the lack of detail and knowledge of the mechanisms that determine resource allocation to each sexual function. Due to this asymmetry, some of the expectations on the evolution of certain patterns of resource allocation are unrealistic.

The evolution of unisexuality in plants (more commonly hermaphroditic; Renner and Ricklefs 1995) may seem unrelated to resource allocation. However, resource allocation is at the core of the theory developed to explain the evolution of dioecy (populations of separate male and female individuals), which requires the evolution of unisexual morphs that replace the cosexual individuals (hermaphrodites) in a population (Bawa 1980; Charlesworth and Charlesworth 1978; Charlesworth 1999; Charnov 1982; Darwin 1877).

The evolution of dioecious plants from hermaphroditic ancestors requires the appearance and spread of unisexual mutants in a population of hermaphroditic individuals. Much theory has developed to examine the conditions necessary for the invasion and maintenance of an initial unisexual mutant, which can no longer gain any fitness via the lost sexual function, and therefore, most likely would have lower fitness than a hermaphrodite (Ashman 2006; Charlesworth 1999; Charnov 1982). However, by not using resources on a second sexual function, unisexual mutants have a surplus of resources relative to their cosexual counterparts. How these resources are used is at the core of the spread of the mutant. By investing these resources on the one remaining sexual function, a unisexual mutant may attain the necessary fitness to spread in a population, depending on whether sterility of one sexual function is determined by nuclear, cytoplasmic or an interaction of nuclear and cytoplasmic genes (Lewis 1941). The loss of the male function via cytoplasmic mutations (mitochondrial) is common (Saumitou-Laprade et al. 1994; Schultz 1994), and because it is maternally inherited (because it is cytoplasmic), it presents fewer obstacles to the spread of the mutation. Thus, an evolutionary pathway in which populations go through a gynodioecious stage has been recognized as a highly plausible one (Domínguez et al. 1997; Lewis 1941; Maurice et al. 1994; Spigler and Ashman 2012).

While most of the initial models are focused on how resource allocation to the remaining function could result in greater fitness for a unisexual mutant, later models considered more indirect investments of resources that could also yield greater fitness for unisexuals. Among these, the investment in greater resistance (Ashman 2002) and tolerance to herbivores could favour the evolution of dioecy (Avila-Sakar and Romanow 2012). The literature on defence against herbivores in dimorphic species has emphasized only one evolutionary scenario, from which the prediction that males should be less resistant than females arises (Ågren et al. 1999). In a review of the topic, Romanow and I examined critically the empirical evidence that purportedly supports the predicted pattern, considered alternative evolutionary scenarios and proposed a standardized protocol for studies of this topic emphasizing the need to consider the causes of the predicted pattern (Avila-Sakar and Romanow 2012). A more recent review recognized our questioning of the evidence used to propose that greater female defence is a rule in dimorphic species (Johnson et al. 2015). The question of whether males and females of a dioecious species should be equally defended and the mechanisms that would evolve should there be sexual differences in the adaptive value of resistance or tolerance traits still need to be adequately and thoroughly studied in a broader taxonomic sample that represents better the diversity of dioecious species (Ågren et al. 1999; Avila-Sakar and Romanow 2012; Renner and Ricklefs 1995).

To understand the changes in allocation to defence brought about by the evolution of unisexuality, Buckley and I conducted a study in natural populations of the dioecious shrub *Ilex glabra* and found high tolerance to partial defoliation (equal fitness of damaged and undamaged plants), with greater tolerance in females than males when damage occurred during, rather than before or after flowering (Buckley and Avila-Sakar 2003). Contrary to sex allocation theory predictions (Ågren et al. 1999; cf. Avila-Sakar and Romanow 2012), we found that males and females had similar tolerance to full defoliation. In contrast, as expected from the principle of allocation, tolerance decreased as plants devoted resources to fruit maturation (Buckley and Avila-Sakar 2003).

Apart from testing for differences in defence between sexes, our study examined whether allocation to reproduction (as biomass) differed between sexes, since a greater allocation to the reproduction of females is proposed as the root cause for sexual dimorphism (Avila-Sakar and Romanow 2012). Contrary to theoretical predictions (Ågren et al. 1999; Lloyd 1984), we found no differences in reproductive allocation between sexes (Buckley and Avila-Sakar 2003). As stated in our review (Avila-Sakar and Romanow 2012), one problem when considering the evolution of unisexuality is assuming that the *only thing* that changes during the evolution of separate sexes is the allocation to reproductive structures, despite increasing evidence of dimorphism in physiology that may result in the neutralization of unitary differences in the resources necessary to produce offspring through the male and female function.

Factors That May Affect Plant Tolerance: Ontogeny, Phenology and Abiotic Factors

Ontogeny – Three Studies with Arabidopsis thaliana *and* Trichoplusia ni

One of the lingering questions regarding studies that were not able to find negative effects of damage on fitness in several species was whether the timing of damage with respect to the developmental stage or the onset of reproduction could influence the susceptibility of fitness to change with damage. In short, does compensatory ability change throughout the lifecycle of a plant?

From the point of view of resource allocation, clearly, a young plant would have fewer organs, and overall, less tissue that would function as a source of resources (C, nutrients, light, water) within the plant compared to an older, mature plant with well-developed root and shoot systems (Boege and Marquis 2005). Similarly, plants with extra sinks such as flowers and developing fruits with seeds actively demanding resources could potentially be more susceptible to changes in fitness due to losses of leaf area to herbivores.

I devoted part of my research programme to answering these questions using a convenient plant-herbivore model system with *Arabidopsis thaliana* as the model plant and *Trichoplusia ni* as the model herbivore. One important advantage of using *A. thaliana* for these studies is that lifetime seed production can be estimated with reasonably good precision and that this measure represents individual fitness attained via the male and female functions because seeds are produced through self-pollination. Instead of only using the two most commonly used genotypes of *A. thaliana* (Col and Ler), I decided to use genetic families that would represent at least some of the variation found in natural populations of this species. Fortunately for me, 30 such genetic families (formerly designated as ecotypes and later as accessions) had been characterized for resistance to *T. ni* in a study that attempted to map resistance traits to particular QTLs and found a locus that accounted for a large percent of the variation in resistance to *T. ni* among the accessions studied, and was, therefore, named TASTY (Jander et al. 2001).

Through these studies, we found that tolerance was greater at the reproductive stage than at an early vegetative stage (Hoque and Avila-Sakar 2015; Tucker and Avila-Sakar 2010), as predicted on the basis of the greater availability of meristems and tissues to garner and store resources that could be remobilized after damage on reproductive plants, which are usually larger than vegetative plants (Boege and Marquis 2005). In our first study in this system, we also found that tolerance correlated positively with inflorescence biomass, a result consistent with findings that the contribution of the inflorescence to lifetime photosynthesis in *Arabidopsis* can be very high (36–93%) (Bazzaz et al. 1979; Earley et al. 2009; Galen et al. 1993). Interestingly, we also found that growth rate slowed down as plants reached the flowering stage. Thus, tolerance was greater at a stage at which plants grew more slowly, as predicted by the GRM and EGRM. This first study used only three accessions (CS20, CS1092 and CS6180, also known, respectively, as Ler-0, Col-0 and Shahdara) which were chosen because they represented a range of resistance levels with Ler-0 being the least resistant and Shahdara the most resistant, and also because Ler-0 and Col-0 have been the most widely studied accessions of *A. thaliana*. This first study did not show any cost of reproduction on tolerance or vice versa: plants were able to compensate for damage despite using resources also for the production of flowers and the initiation of fruits.

In the next two studies (Hoque and Avila-Sakar 2015; Kornelsen and Avila-Sakar 2015), we simultaneously assessed constitutive resistance and tolerance, used more accessions and included a later stage that would presumably represent a time at which more resources were committed to reproduction. We named it the first-fruit stage and defined it as the developmental stage at which plants had one ripe fruit, and the main inflorescence had 10–15 fruits. Usually, at this stage, plants also had several secondary inflorescences, each with initiated fruits and flower buds still being produced and several flowers opening every day.

In Hoque's study, which was conducted in a greenhouse, with a total of 420 plants (7 accessions, 3 ontogenetic stages, 20 plants per accession-ontogenetic stage combination), we found that plants damaged at an early vegetative stage undercompensated, while those damaged at the flower and fruit stages equally compensated (Hoque and Avila-Sakar 2015). This study confirmed the positive ontogenetic trajectory of tolerance found in our earlier study, and somewhat to our surprise, found that resistance also increased with ontogeny. We also found a trade-off between resistance and tolerance but only at the earliest ontogenetic stage. This result was contrary to our expectation of finding stronger trade-offs during the reproductive stage based on the purported greater shunting of resources towards reproduction rather than defence. Thus, we can conclude that *A. thaliana* at the reproductive stage had the means (structures and physiological capacity) both to reproduce and to defend better than younger ones. We also detected a cost of tolerance as measured by a negative true (corrected) covariance between tolerance and fitness in the absence of damage (Mauricio et al. 1997), but only at the early vegetative stage, which is consistent with the trade-off found at the same ontogenetic stage.

Interestingly, in Kornelsen's study, while we found that resistance increased through ontogeny, we did not see the same ontogenetic trajectory for tolerance (Kornelsen and Avila-Sakar 2015). In this study we detected the costs of tolerance regardless of the ontogenetic stage but did not find a trade-off between resistance and tolerance. Moreover, we found a positive association between tolerance and both the overall size of plants (total biomass at senescence) and allocation to root growth. Two differences with Hoque's study that may have influenced our findings are that plants in this experiment grew on fluorescent light shelves inside a laboratory rather than in a greenhouse and also that we used fewer replicate plants per accession-ontogenetic stage combination (10 instead of 20) due to space constraints in our growth shelves. Lastly, while the number of accessions was similar between both studies (seven versus eight), the accessions used were different, and although they should be roughly equally representative of the variation of defence responses of the species, some of the genotypes in this set may happen to have different ontogenetic trajectories of defence traits by chance.

A separate experiment (unpublished) conducted in the greenhouse, but during the winter months and using mostly different accessions from those in either of the previously described studies shows how the defence responses of plants can be influenced both by genetics (differences among accessions) and growth conditions (light, temperature; Hoque 2013). In this experiment, we found no effects of ontogeny on either resistance or tolerance. While we presented these results at several scientific meetings, when we submitted our manuscript for publication, the reviewers suggested that we removed this experiment (because it did not help convey a clear message). In hindsight, the message is that the growth conditions matter for the detection of ontogenetic trajectories of resistance and tolerance and speaks to the plasticity of both kinds of defence traits.

One unexpected finding, especially in a species presumed to have low genetic diversity because most fruits are produced by selfing (especially in controlled environments), was an enormous variation in seed production, even in undamaged plants of the same accession grown within in a greenhouse or laboratory.

A few important notes are that in all these experiments, we used larvae to produce damage and elicit responses and after larval feeding, we proceeded to adjust the level of damage according to the desired levels. We achieved very accurate and precise levels of damage.

In summary, these studies showed that both tolerance and resistance increase from the early vegetative to the fruit-maturation ontogenetic stages, that a trade-off between tolerance and resistance and costs of tolerance can be detected at the vegetative stage, but not at later reproductive stages, and that allocation to root tissues is important for tolerance. This latter result complements findings from other research groups that show plants are able to translocate carbon and nitrogen compounds to the root upon herbivore attack, and then translocate them back to the shoot during regrowth (Orians et al. 2011; Schwachtje et al. 2006).

Abiotic Stress

More recently, I started a series of collaborative studies on the simultaneous responses of plants to salinity and herbivory, two stress factors that elicit responses through partially overlapping signalling pathways (Rejeb et al. 2014). This is an understudied topic that addresses a more realistic situation: plants in natural and managed populations are frequently under stress due to multiple biotic and abiotic factors. In particular, salinity is common in natural ecosystems and can be a severe problem in many agroecosystems, with 20–50% of agricultural land affected. An experiment with *Brassica juncea* (Indian mustard) showed that plants growing under salinity had greater constitutive and induced resistance to larvae of the generalist herbivore *T. ni*. However, tolerance was unaffected by soil salinity (Renault et al. 2016). We performed a similar experiment in *Glycine max* (soybean) to study the simultaneous effects of N fixation and salinity on defence (Avila-Sakar et al. 2018). We found no effects of N fixation (N availability) on resistance or compensatory ability. However, in contrast to *B. juncea*, in this species, plants growing under salt stress had lower constitutive and induced resistance. We could not assess compensatory ability (measurement based on seed production) in salt-stressed plants because, with one exception, salt-stressed plants produced no seeds. Apart from addressing the understudied topic of plant responses to simultaneous biotic and abiotic stress, we aimed at improving our understanding of the crossed responses to salinity and herbivory in species not adapted to salinity.

Difficulties and Remaining Questions

One of the main difficulties in the estimation of tolerance is the need for clones of the same genotype or full-sib genetic families so that the genetic contribution to the relation between fitness and the intensity of damage can be determined as a norm of reaction (Pilson 2000). Each family provides one estimate of tolerance, and given that there may be differences in tolerance among genotypes, it would be ideal to maximize the number of clones or genetic families sampled from a population so as to characterize tolerance in that population with high confidence. Even using few replicates per genetic family, this usually calls for large experiments with several hundreds of plants. Thus, the addition of factors of interest (ontogenetic stage, nutrient availability, etc.) in the experimental design, even at the minimum of two levels each, soon becomes impracticable. In addition, using a small number of clones or genetic families results in low statistical power to test hypotheses.

As a way to refocus on the capacity of each individual plant to recover from herbivore damage, and at the same time not lose the statistical power provided by replication at the individual level, I have resorted to the estimation of individual compensatory ability (Tucker and Avila-Sakar 2010), a measure of the fitness of an individual subjected to herbivory relative to the typical fitness (median or mean value) of undamaged individuals (in the same genetic family, if this information is available).

One of the remaining questions after this series of studies is whether induced resistance is also greater in (later) reproductive stages of development compared to early vegetative stages. We did not include induced resistance in our studies in *Arabidopsis* because, as mentioned above, we would have needed to perform either a separate experiment or have at least twice the number of replicates in our studies. Futures studies of the ontogenetic trajectory of induced resistance are clearly warranted.

Despite our efforts to measure variables that would help us elucidate the mechanisms of tolerance, we still did not get to the level of detail needed for a thorough understanding of them, at least with enough detail as we now have for resistance mechanisms. Our results lend support to findings from other studies that show how C and N are translocated to the root upon herbivory of the shoot (Gomez et al. 2010; Schwachtje et al. 2006). Together, results from different studies allow us to understand that apart from the particular molecular mechanisms that allow for compounds with C and N to be translocated from the root to the shoot, plants need root cells (and tissues) in which to place these compounds. Thus, plants with more root biomass should have greater compensatory ability than those with less.

One other question related to mechanisms of translocation that has received little attention is whether particular anatomical traits are more conducive to translocation, for instance, the degree of physiological integration sensu Watson and Casper (1984). This question came up when studying the effects of different spatial patterns of damage in *Cucurbita pepo* subsp. *texana* (Avila-Sakar et al. 2003). The general lack of effects of the spatial pattern of damage along the shoot, plus the high level of compensatory ability seen in this species (including overcompensation for pollen performance), suggested that plants were able to readily translocate resources from undamaged parts (whether on the shoot or root) and thus pointed towards plants having a highly interconnected network of vascular tissues. While we did not perform the anatomical study to verify this hypothesis, another species in the genus *C. foetidissima* does have a highly interconnected vascular system (Vasudeva Rao and Iyengar 1983), and therefore, I think it is likely to be so in *C. pepo* subsp. *texana*. Two different survey studies could help establish the generality and importance of the pattern of vascular network on the compensatory ability of plants. One approach is to select a broad sample of species for which compensatory ability is known, and that includes both under- and overcompensating species. One would hypothesize that the level of interconnectedness of the vascular system differs between both groups of species. The converse approach would be to gather a broad collection of species with representatives of those with highly interconnected vascular systems and those with highly sectorial systems. One could then test the hypothesis that equal and overcompensation would be more common in the first group, while undercompensation would be the more common response in the latter.

Costs and Benefits of Defence: Mechanisms

Costs of resistance and tolerance can be assessed by comparing the fitness of individuals that have a trait that confers resistance or tolerance with the fitness of individuals that do not have that trait, all in the absence of herbivores. Lower fitness of the individuals with the trait in question would indicate a cost, and the difference could be interpreted as the magnitude of the cost. If, however, costs are not detected, can we be sure that having the trait in question has no cost? Such a conclusion is difficult to admit given that even if minimal, there should be some construction costs (see below). To understand fully the costs and benefits of resistance and tolerance to herbivore attack, we need to know the mechanics of the costs, what exactly causes a cost, and for that we need to know the mechanisms of resistance and tolerance.

Resistance

Costs of resistance can be direct or indirect: direct costs arise without intermediary from the trait that confers resistance; indirect costs arise from the interaction with another species (Strauss et al. 2002). Direct costs include allocation costs (construction and transport costs of a structure or chemical compound, disruption of biochemical pathways, pleiotropic effects of the genes that determine the resistance trait), costs of avoiding autotoxicity and opportunity costs. For instance, *Datura wrightii* that have glandular trichomes incur a direct cost that results in the production of 45% fewer viable seeds than plants that only produce non-glandular trichomes (Hare et al. 2003). A couple of cases illustrate the costs of avoiding autotoxicity: in *Trifolium repens,* the precursor of cyanide and the enzyme that catalyses the last step in the reaction to produce cyanide are kept in separate cell compartments (Majumdar et al. 2004). In this case, plants incur direct costs in keeping the substrate and the enzyme in separate compartments within cells, plus the actual cases of autotoxicity that may occur during damage due to causes other than herbivores. In addition, plants incur opportunity costs, as some of their resources are expended in secondary metabolite production (precursors, enzymes, vacuoles) could have been otherwise used in growth and reproduction and can be measured as lower fitness of the cyanogenic morphs in the absence of herbivores (Dirzo and Harper 1982). A similar situation occurs in plants of the mustard family (Brassicaceae), which store glucosinolates in separate vacuoles from myrosinase, the enzyme that cleaves the glucose portion of a glucosinolate to produce an aglycone that reorganizes itself into biologically active products such as isothiocyanates and nitriles (Halkier and Gershenzon 2006; Taiz et al. 2015). Indirect costs are also known as ecological costs and include the deterrence of mutualists, favouring greater parasite loads, reduced tolerance of natural enemies (included herbivores) and reduced competitive ability (Heil 2002).

In several studies, scientists have estimated the costs of resistance in terms of the amounts of energy and materials needed to express the resistance trait (including the transcription and translation of genes necessary in the biochemical pathways involved in the expression of the trait), or in terms of the difference in fitness between plants that have the trait in question and those that do not have it. In the latter case, several questions remain: What makes the plants more or less resistant? Is it one or several traits? What is it about the way that a plant uses its resources that translates into a cost as measured by a decrease in fitness? Testing for the role of candidate traits in the resistance of plants and their fitness cost allows for a more detailed understanding of the ecology and evolution of resistance to herbivory. Some of this information would be useful in breeding programmes that intend to use specific traits to provide crops with greater resistance to pests. If breeding such trait into crops will have negative effects (costs) on yield, plant breeders could prioritize their efforts to work on traits that would incur the lowest costs to the plants, and therefore, the lowest negative effects on yield.

More importantly, while we may be able to estimate the construction costs of a certain trait (in ATP units or actual biomass required to express the trait), ultimately, the fitness cost (the reduction in fitness due to expressing the trait) is what will determine the adaptive value and evolutionary perdurance of the trait in a particular set of circumstances. A similar argument can be made about tolerance and tolerance traits.

Tolerance

As with resistance, the costs of tolerance can be direct or indirect. To estimate direct costs of tolerance, the mechanisms and traits that confer tolerance must be known. For a long time, the mechanisms and traits involved in tolerance were elusive. It was hypothesized that traits and conditions that allowed for fast relative growth rates would favour tolerance, including adequate availability of nutrients, water and light, whether determined more by abiotic conditions or biotic interactions. Tolerance would also be favoured by traits that allowed for the continuation or resumption of growth after damage, such as meristem availability and the capacity to store and mobilize resources. Thus, apart from meristems and their release from apical dominance, no concrete structures, biochemical pathways or cellular processes that would result in regrowth were proposed, let alone those that would explain that plants could fully compensate or overcompensate for damage (Núñez-Farfán et al. 2007).

As mentioned previously, a physiologically based model for the responses of plants to grazing actually predicted a greater compensatory ability for plants with lower relative growth rates (Hilbert et al. 1981). Interestingly, the authors did not propose any cellular or organismal processes that could link slow growth with greater compensatory ability, which, combined with such a counterintuitive prediction and differences in the terminology used, may explain partly why so little attention has been paid to this model. However, further examination of the model shows that the link between greater compensatory ability and slow growth arises from the fact that compensatory ability (and tolerance) is defined in terms of the fitness of damaged individuals relative to the fitness of undamaged individuals, whose gains in biomass per unit time (correlated to fitness) become smaller as the growth rate of an individual decreases. Ultimately, equal compensation and overcompensation are predicted to occur only if plants increase their RGR in response to damage (Avila-Sakar and Laarakker 2011).

Recently, endoreduplication and the resulting increased ploidy of cells – endopolyploidy – were found to be associated with plant tolerance to herbivory (Ramula et al. 2019; Scholes and Paige 2015). Work on *A. thaliana* revealed that overcompensation after apical meristem damage is associated with elevated gene expression (facilitated by endopolyploidy) of glucose-6-phosphate-1-dehydrogenase (*G6PD1*), a central enzyme in an NADPH-producing metabolic pathway (Siddappaji et al. 2013). Additionally, two invertases (enzymes involved in sucrose hydrolysis and transport) have been found to enhance compensatory ability (Schultz et al. 2013; Siddappaji et al. 2015). Although exciting, these novel mechanisms of tolerance have only been documented in three annual herbaceous (model) species: tobacco, tomato and *A. thaliana* (two accessions). The generality of the role of endoreduplication and/or upregulation of specific genes in response to foliar damage (not only to apical meristem damage) as a mechanism that facilitates plant tolerance to herbivory warrants further assessment, especially in woody plants, where the prevalence of endoreduplication has not been thoroughly investigated (only a handful of species have been assessed; see Barow and Meister 2003). Further research on the cellular and molecular bases of processes that lead to enhanced compensatory ability promises to bring our understanding of the mechanisms of tolerance at least to the same level of detail as we now have for the mechanisms of resistance.

Tolerance and Growth

Importantly, all of the traits proposed or shown to be linked to tolerance are traits involved in growth. While both resistance and tolerance are considered defence traits (sensu Karban and Baldwin 1997) because plants with the traits have greater fitness than plants without the traits in the presence of herbivores, there is a fundamental difference between both kinds of traits: Resistance traits can be expressed without the need of herbivore attack (constitutive defence). In contrast, tolerance traits are only expressed upon damage by herbivores (induced defence).

Tolerance traits are all linked to growth, and ultimately, it is through growth that plants reach a reproductive phase. In addition, by providing the infrastructure needed to produce reproductive structures, the amount of vegetative growth is an important determinant of the reproductive output of a plant. Thus, except for the rare, inviable mutant that does not live past some very early embryonic stage because it fails to establish its first growth axis through the generation of the first root and shoot apical meristems, all plants will have traits that can confer them with some degree of tolerance of herbivores. In short, if a plant can grow, it can tolerate some degree of herbivory.

Given the seemingly inextricable link between tolerance and growth, direct costs of tolerance should be lower than those of resistance because many of the traits that confer tolerance of herbivory to a plant are not optional: a plant that grows makes meristems and has mechanisms to mobilize resources between the root and the shoot, and it has a certain amount of parenchyma in the stems and roots that can serve as storage tissue.

Of all the suggested mechanisms of tolerance, perhaps endoreduplication is the least directly related just to growth, understood as the simple addition of cells, tissues and organs. This said, endoreduplication is important both for the rate of growth (and expansion of the cells) and also for the final differentiation of cells, such as trichomes. Interestingly, this particular case also exemplifies how endoreduplication is important for resistance, as glandular trichomes are able to produce large quantities of secondary metabolites and store them in enlarged cells thanks to endoreduplication. Thus, endoreduplication is important for both tolerance and resistance against herbivores!

Factors That Affect the Costs of Resistance and Tolerance

A thorough understanding of the factors that affect the costs of resistance and tolerance requires a similarly thorough understanding of the traits involved in defence and any pleiotropic functions they may have. Given our better understanding of resistance traits, elucidating the factors that affect their costs should be more straightforward than for tolerance traits, as evidenced by several reviews of the topic (Cipollini et al. 2014). Evidence of costs of tolerance is lacking, so there is work to be done in this area. For both resistance and tolerance, one could argue the constitutive level of defence traits should reflect its cost. In this view, since tolerance is expressed as a response to damage, it could be expected to be generally less costly than resistance. Moreover, given the primary involvement of tolerance-related traits in growth, their cost should be low (i.e. in the absence of damage, they are contributing to fitness via growth). On the other hand, by restricting growth to its occurrence mostly after damage, as in *Ipomopsis aggregata* (Paige 1999), it could be argued that high tolerance (including the ability to overcompensate) has great costs. Thus, we could predict that genotypes and species that generally overcompensate experience the highest costs of tolerance. Here, I analyse three sources of variation for tolerance and its costs.

Life History For annual plants, one bout of herbivory and the consequent loss of tissue may have more detrimental consequences to its fitness than for perennial plants. Perennials invest in survival, so removal of their tissues may delay their peak reproduction, but they do not have the same constraint as an annual which is limited by the impinging arrival of an unfavourable season that will kill them, truncating their reproductive stage at best, or at worst, impeding it altogether. It would seem that being tolerant is less important for perennials because they have future opportunities for reproduction. However, the likelihood of such future opportunities materializing depends on an individual's investment in survival of its vegetative structures (soma). High investment in survival may imply a greater per unit value of vegetative tissues, in which case investing in tolerance would bring greater returns, thus lowering its fitness cost. Investing in tolerance would be a high-cost-high-benefit strategy if it ultimately increases individual survival.

It is difficult to hypothesize on the costs of tolerance in perennials (and also in annuals) without knowing for sure the mechanisms of tolerance. In perennials, the production of meristems might have low construction costs, as plants do this as a part of their growth. Investing in parenchyma and storage tissue might be also part of the growth, and may be modified with little cost. However, as stated above, the suppression of those meristems and not using the stored reserves may signify large opportunity costs.

Development In annuals, tolerance should be more costly during earlier stages of development because (1) there are fewer source organs to supply the resources needed for tolerance and (2) growth should have allocation priority. Indeed, these are the same reasons to expect plants to be more tolerant at later than earlier stages of development (Boege and Marquis 2005) – an expectation for which there seems to be reasonable support, as mentioned above, although some species have the opposite ontogenetic trajectory of tolerance (Lorca et al. 2019). Interestingly, plants may have the same ontogenetic trajectory for resistance, but as shown in our work with *Arabidopsis*, it seems that larger plants are able to cover the direct costs of defence through both resistance and tolerance better at later ontogenetic stages, and consequently not suffer any negative fitness consequences of investing in both modes of defence (Hoque and Avila-Sakar 2015).

Physiology If the construction costs of a trait are determined by the availability of resources, the capacity of plants to acquire those resources and the availability of enzymes and cellular structures required for a given biochemical pathway or process that produces said trait, then it would follow that individuals with more efficient biochemical capacity, greater availability of the needed cellular structures and greater capacity to acquire and use resources would incur lower costs of defence. This argument seems more applicable to resistance traits (trichome, secondary metabolite production, etc.), but could also be explored for tolerance traits.

Conclusion and Future Directions

In summary, there have been important advances in the field of tolerance to herbivory in the last two decades. Namely, tolerance is now recognized as a mode or strategy of plant defence rather than a methodological artefact. Clearly, we are still far from understanding the mechanisms of tolerance at the level that we understand those of resistance, but the recent incursions into the role of endoreduplication in overcompensation and tolerance in general seem very promising. Indeed, I would suggest that the main ways in which we can advance our understanding of the mechanisms of tolerance is by expanding our studies to plants in wild populations and investigating further into the role of endoreduplication in enhancing the expression of certain genes that favour tolerance. As we unravel these mechanisms in *Arabidopsis* and other model herbaceous plants, it is also time to start investigating whether these mechanisms operate also in woody plants, both temperate and tropical.

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Chapter 4
Toward a Unifying Quest for an Understanding of Tolerance Mechanisms to Herbivore Damage and Its Eco-Evolutionary Dynamics

Iván Darío Camargo

Abstract The evolutionary and ecological study of tolerance to herbivory has been centered in its operational definition. In spite of the critical role of the operational definition of tolerance that has permitted its evolutionary modeling, a causal understanding of its mechanisms that allow a universal approximation to look for difference across populations and species is mandatory. The author proposes a research program for tolerance mechanisms whose focus is not to produce long-term predictions of its evolutionary change but to determine the causes and objects of selection. The reward for this study framework is a more precise understanding of the adaptations driving by herbivory, in contrast to other selective pressures behind the evolution of tolerance lineages. Additionally, it can throw much light on the possibility that specific tolerance mechanisms within plant groups affect plant-herbivore coevolution by imposing selection on herbivores.

Keywords Relative growth rate · Growth determinants · Net assimilation rate · Leaf weight ratio · Specific leaf area · Tolerance · Plasticity to defoliation · General vigor · Fitness reaction norm · Underlying trait reaction norm

The Current Meanings of Tolerance

Two main concepts about tolerance have dominated plant defense theory: tolerance as a pattern and tolerance as a mechanism. As a pattern, tolerance is the plasticity (i.e., the slope with an amount and sign) of the fitness reaction norm of a genotype in response to a gradient of damage (Simms 2000; Stowe et al. 2000, p. 567).

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Therefore, a genotype can be utterly homeostatic to damage (i.e., maintaining fitness across the damage gradient) or can be plastic (i.e., being either an undertolerant or overtolerant genotype). This pattern view differs from the mechanistic view of tolerance, in which it is a “capacity of plants” to survive and reproduce in the face of herbivore damage (Painter 1958; Rosenthal and Kotanen 1994; Strauss and Agrawal 1999; Stowe et al. 2000). This capacity has been showed (theoretically and experimentally; Nuñez-Farfán et al. 2007) due to pronounced plasticity to defoliation in another phenotypic trait, less visible and more distantly connected to fitness (i.e., an underlying trait, UT; Alper and Simms 2002; see Table 4.1 for all abbreviations used).

The above concepts have been beneficial to model plant tolerance to herbivory (reviewed in Fornoni et al. 2003). Yet, there is an epistemological unlink between the operational definition and the causal mechanisms that generate the patterns of tolerance observed. This unlink is possibly one of the most important reasons for the lack of a mechanistic basis to model tolerance (but see Rosenthal and Dirzo 1997; Juenger and Bergelson 2000; Weis et al. 2000; Juenger et al. 2000; Camargo et al. 2015; Camargo 2017). A particular fitness reaction norm to whatever environmental stress is achieved through the interaction of many types of underlying plasticity at many levels within the whole organism (Pigliucci 2001). This multivariate causal nature of tolerance implies the further use of conceptual and methodological tools

Table 4.1 Abbreviations, its definitions, and units

Abbreviation	Trait	Definition	Unit
A_a	Rate of photosynthesis	Net CO_2 uptake/leaf area/time	mol m^{-2} s^{-1} or mol m^{-2} day^{-1}
C_p	Carbon concentration of a plant	Carbon/total plant dry mass	mmol C g^{-1}
CUE	Carbon use efficiency	Fraction of daily fixed C used for growth	mol mol^{-1}
LAR	Leaf area ratio	Leaf area/total plant dry mass	m^2 kg^{-1}
LD	Leaf density	Leaf dry mass/leaf volume	g ml^{-1}
LNC	Leaf nitrogen concentration	Leaf nitrogen/leaf area	mol N m^{-2}
LWR	Leaf weight ratio	Leaf dry weight/total plant dry weight	g g^{-1}
NAR	Net assimilation rate	Increase in total plant dry mass/leaf area/time	g m^{-2} day^{-1}
PNUE	Photosynthetic nitrogen use efficiency	Photosynthesis/leaf organic nitrogen/time	mol CO_2 mol^{-1} N s^{-1}
RGR	Relative growth rate	Increase in plant mass/total plant dry mass/time	mg g^{-1} day^{-1}
SLA	Specific leaf area	Leaf area/leaf dry mass	m^2 kg^{-1}
UT	Underlying trait	A trait distantly connected to fitness	Not specified

that can explain the genetic association between traits involved in mechanisms and the operational definition of tolerance.

The author aims not to review putative tolerance traits, which had received exhaustive reviews (Rosenthal and Kotanen 1994; Strauss and Agrawal 1999; Tiffin 2000; Stowe et al. 2000; Fornoni et al. 2003). Instead, he uses compensatory growth to illustrate the complex relationships of the multivariate causal nature of tolerance to herbivory. Compensatory growth is the first response after damage (Hilbert et al. 1981; McNaughton 1983; Strauss and Agrawal 1999; Stowe et al. 2000; Tiffin 2000; Camargo et al. 2015). It is composed of several plastic phenotypic changes in different traits, which can determine the pattern of change in RGR after defoliation (Oesterheld and McNaughton 1988; Trumble et al. 1993; Strauss and Agrawal 1999; Stowe et al. 2000; Tiffin 2000). Plastic responses of compensatory growth include several traits as an increasing photosynthetic rate (Caldwell et al. 1981; Wallace et al. 1984); changing the allocation pattern to increase the production of new leaf area (McNaughton and Chapin 1985) or nutrient uptake (Ruess et al. 1983; McNaughton and Chapin 1985), and improving plant water status (Toft et al. 1987). Table 4.2 presents some putative traits that had received some attention in the last years.

Table 4.2 Some new putative traits which potentially may promote tolerance to herbivore damage, reported in recent studies

Trait	System	Refs.
Grow determinants		
NAR (growth per unit leaf area)	*Chamaedorea elegans*, *Stipa krylovii*, *Datura stramonium*	Anten et al. (2003), Van Staalduinen and Anten (2005), and Camargo et al. (2015)
Constitutive levels of SLA (leaf area per unit leaf biomass)	*Asclepias incarnata*	Agrawal et al. (2009)
LWR (leaf weight ratio)	*Datura stramonium*, *Poa bulbosa*	Camargo et al. (2015), Van Staalduinen et al. (2010), and Dobarro et al. (2012)
Biomass allocation		
Allocation of new mass to the production of lamina tissue	*Chamaedorea elegans*	Anten et al. (2003)
Allocation to root growth for higher potential future reproduction	*Solanum carolinense*	Wise et al. (2008)
An accumulation of carbon reserves in roots based on pre-existing glucose in below-ground tissues to increase resource acquisition	*Ruellia nudiflora*	Rivera-Solís et al. (2012)
Constitutive biomass allocation to stems	*Populus tremuloides*	Stevens et al. (2008)
Increased allocation to stems	*Populus tremuloides*	Stevens et al. (2008)
Stem biomass at senescence	*Arabidopsis thaliana*	Tucker and Avila-Sakar (2010)
Reduction of shoot biomass	*Ipomea purpura*	Atala and Gianoli (2009)

Table 4.2 (continued)

Trait	System	Refs.
Large seed mass	*Aesculus californica*	Mendoza and Dirzo (2009)[a]
Physiological integration of roots and shoots	*Nicotiana tabacum*	Kaplan et al. (2008)
Morphological		
Numbers of reserve buds	*Fragaria virginiana*	Ashman et al. (2004)
New lateral bud activation without removal of apical meristems	*Salix gilgiana*, *Salix eriocarpa*, and *Salix serissaefolia*	Utsumi and Ohgushi (2007)
Clonal integration	*Bromus ircutensis* and *Psammochloa villosa*	Liu et al. (2009)
Inducibility of flower production	*Ipomopsis aggregata*	Irwin (2009) and Wise et al. (2008)
Production of high-quality new leaves	*Salix eriocarpa*	Utsumi et al. (2009)[b]
Photosynthetic		
Compensatory photosynthesis to the leaves	*Leymus chinensis*	Zhao et al. (2008)[c]
Constitutive photosynthetic efficiency	*Avena barbata*	Suwa and Maherali (2008)
Chlorophyll fluorescence	*Aextoxicon punctatum*	Madriaza et al. (2019)

[a] The surrogate of fitness in this study is survival

[b] The first study showing tolerance putative traits unchaining a possible coevolutionary response

[c] But see Nabity et al. (2009)

The author’s intention is then a critical meta-evaluation (Pigliucci and Kaplan 2006) of the concepts used and, from here, to propose some methods within the quest for a better causal understanding of tolerance. The author’s focus is on internal mechanisms of tolerance because external abiotic factors that mediate internal mechanisms and affect the expression of tolerance have received thoughtful reviews (Wise and Abrahamson 2005, 2007). However, the author offers an approach to study how external factors would mediate internal mechanisms. The author contends that the proper articulation of the concepts “putative,” “component,” and “mechanism” of tolerance is not a matter of semantics but is one of the reasons why we could not explain the patterns of tolerance satisfactorily.

Plasticity of Fitness vs. No Fitness Traits and the Identification of Tolerance Components

Plasticity of morphological and physiological traits is distinct from plasticity in fitness traits and potentially may contribute to or control plasticity in fitness (Bradshaw 1965; Sultan 1995; Richards et al. 2006, e.g., Sultan et al. 1998). In this context, traits involved in mechanisms of tolerance to herbivore damage could be constitutive[1] phenotypic responses that occur before damage or induced (plastic) phenotypic responses after damage (Karban and Baldwin 1997). If natural selection generally acts to maintain high levels of fitness across environments, the resulting most favorable reaction norm for fitness may be complete tolerance, an invariable or flat (i.e., fitness homeostasis; Hoffmann and Parsons 1991; Rejmánek 2000). Thus, it is possible to observe plasticity for a specific morphological or physiological trait and lack of plasticity for a fitness component (Richards et al. 2006; see Fig. 1B in Alper and Simms 2002). On the contrary, this may be an indication that plasticity in the underlying morphological or physiological trait possibly is the result of natural selection (Bradshaw 1965; Sultan 1987, 1995; Pigliucci 2001; Alper and Simms 2002); hence, the trait is a tolerance component. However, phenotypic plasticity cannot automatically be assumed to be adaptive. It is an adaptive plasticity that is of particular importance for understanding if a trait is a tolerance component (see Fig. 1 in Alper and Simms 2002).

Selection gradient analysis is one of the best available tools to infer which traits can predict tolerance to herbivory (e.g., Juenger and Bergelson 2000). Notwithstanding, plastic genotypes with the highest global fitness averaged across environments (i.e., the highest general vigor), rather than the highest fitness in each environment (Relyea 2002), are required for the evolution of adaptive plasticity (van Kleunen and Fischer 2005). Analyses in which the fitness of individuals is regressed on the trait of interest separately for different defoliated environments are robust in determining if the putative-plastic response per se would be beneficial. Still, they do not unequivocally prove that the plastic genotypes are under selection (e.g., Juenger and Bergelson 2000). It occurs because the most plastic genotypes could not produce as extreme trait values as the less plastic specialist genotypes in each environment (see Fig. 2 in van Kleunen and Fischer 2005) or when the putative trait is not linearly related to fitness (Stearns and Hoekstra 2000; Fornoni and Núñez-Farfán 2000; van Kleunen and Fischer 2005).

Stowe et al. (2000) emphasized that distinguishing between general vigor and tolerance may be necessary to understand the evolutionary response of plants to consumer-imposed selection. While considerable effort to uncover tolerance mechanisms (reviewed in Strauss and Agrawal 1999; Stowe et al. 2000; Tiffin 2000; Fornoni et al. 2003) was focused on the relationship between the plasticity of underlying traits and plasticity in fitness (e.g., Wise et al. 2008; Hochwender et al. 2000; Juenger and Bergelson 2000; Strauss et al. 2003), other properties of the underlying reaction norms have not been taken into account.

Plasticity is not the unique property of a linear reaction norm. The across-environment expected trait value (reaction norm elevation) of an underlying trait

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[1] Note that what we call a *constitutive response*, by having a strong correlation with tolerance, is the product of an allocation response before damage to enhance both functions (mainly plastic responses to damage), the potential for future reproduction (e.g., Wise et al. 2008), or to increase organ resource acquisition (e.g., Rivera-Solis et al. 2012). Only when we prove these functions are the response constitutive and not just when we find the statistical association, which might be spurious

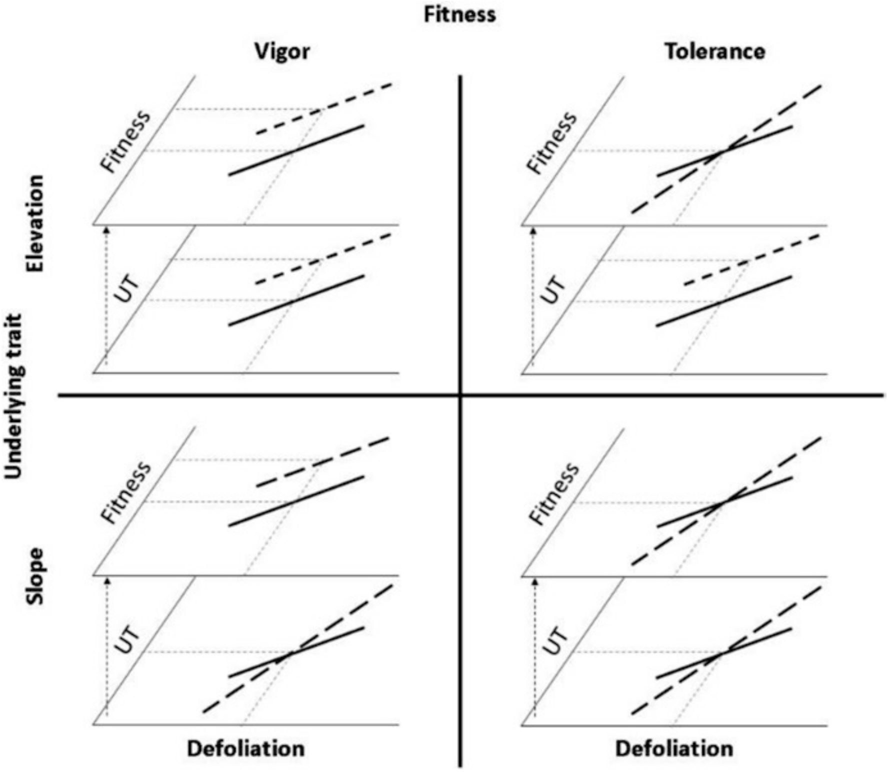

Fig. 4.1 Four scenarios of the relationship between the properties (slope and elevation) of an underlying trait (UT) reaction norm to damage and the properties (tolerance and vigor) of the fitness reaction norm. Two genotypes are depicted (solid and dashed lines), which vary in slope and elevation. Upper left, a UT increase in elevation for a genotype (dashed line) is related to an increase in the general vigor (elevation) of the fitness reaction norm. Lower left, a UT increase in plasticity for a genotype (slope of dashed line) is related to an increase in the general vigor. Upper right, a UT increase in elevation is related to an increase in tolerance (fitness plasticity). Lower right, a UT increase in plasticity is related to an increase of tolerance

could have fitness consequences, too (Scheiner 1993a, 2002; Stinchcombe et al. 2004; Gavrilets and Scheiner 1993). However, these consequences not only would impact the plasticity of fitness but its general vigor (reaction norm elevation of fitness, Stowe et al. 2000). In other words, to uncover tolerance components, it is necessary to assess the relationship between both properties of reaction norms, the slope and elevation of underlying traits and fitness. Yet, this had not been accomplished in the literature; Fig. 4.1 is a graphical model of such analysis. Two independent equations for three underlying traits represent the statistical version of the graphical model in Fig. 4.1, as follows:

$$\overline{W}_j = \text{Constant} + \alpha_0 \overline{X_0}_j + \alpha_1 \overline{X_1}_j + \alpha_2 \overline{X_2}_j + \beta_0 plX_{0j} + \beta_1 plX_{1j} + \beta_2 plX_{2j} \tag{4.1}$$

$$plW_j = \text{Constant} + \alpha_0 \overline{X_0}_j + \alpha_1 \overline{X_1}_j + \alpha_2 \overline{X_2}_j + \beta_0 plX_{0j} + \beta_1 plX_{1j} + \beta_2 plX_{2j} \quad (4.2)$$

The equations above regressed relative values of a fitness component grand mean (i.e., general vigor, $\overline{W}_j$, averaged over control and defoliated plants) (Weis and Gorman 1990; Stinchcombe et al. 2004), their plasticity (i.e., tolerance, plW_j), over the estimates of underlying trait, means (i.e., elevation of the reaction norm, $\overline{X}_j$), and plasticities (i.e., steepness of the reaction norm, plX_j) measured. The model might contain interaction terms (not shown in 4.1 and 4.2). For instance, a positive interaction term would indicate that the increase in vigor due to plasticity in an underlying trait increases with the elevation of its reaction norm (Eq. 4.1); the same would occur for tolerance (Eq. 4.2; for a thoughtful discussion of interaction terms in selection models; see Philips and Arnolds 1989).

Finally, we need a working definition for a tolerance component. Is it an underlying trait affecting both vigor and tolerance (models 4.1 and 4.2) or just tolerance (model 4.2)? Camargo (2017) which proposed that to be identified as a tolerance component, a liberal rule would imply that an underlying trait should predict at less tolerance (model 4.2). A more conservative rule states that a tolerance component must predict both vigor (model 4.1) and tolerance (model 4.2). While predicting the last one is an indication of the usefulness of the underlying trait to cope with the adverse effects of herbivory damage within a generation, predicting the former is an indication of the underlying trait capacity for evolutionary change driving by variation in its reaction norm (Example 4.1).

Example 4.1: Identification of Tolerance Components

A fitness reaction norm to defoliation depends on complex mechanisms with multiple interacting underlying reaction norms, which can influence both vigor and tolerance or just tolerance. The plasticity observed in response to damage could be beneficial for vigor but detrimental for tolerance. Only when we observe the relationships among traits and between traits and the environment in which they are developing that we can both explain satisfactorily the patterns of tolerance observed and identify a tolerance component accurately. For instance, plants of *Datura stramonium* are more tolerant of defoliation when growing in high light, in contrast to low-light environments (Cisneros-Silva et al. 2017). While in low-light environments, there are no traits that can explain the observed lower pattern of tolerance, the high-light tolerance to defoliation is the result of a positive correlation of multiple traits with tolerance that buffer the negative effects of some of the traits (Camargo 2017). That is, the negative effects on tolerance by plasticities of both leaf number (NL) and total leaf area (TLA) and the height of chlorophyll content index (CCI) are buffered by the positive effects of the plasticity of CCI and the height of TLA (Table 4.E1, Fig. 4.E1a, b).

A genotype of *D. stramonium* confronts an evolutionary disjunctive if the increase in general vigor due to plasticity in NL (i.e., a higher increase in defoliated environments, Fig. 4.E1.c) results costly increasing the susceptibility to herbivory (negative effects of NL on tolerance, Table 4.E1, Fig. 4.E1a). Notwithstanding, tolerance is the product of several traits that counteract the negative effect of plasticity in leaf number (Fig. 4.E1a, b).

Table 4.E1 Stepwise regressions of properties (plasticity and vigor) of fitness component reaction norms on properties (plasticity and height) of growth component reaction norms in *Datura stramonium* growing in a high-light environment

	Seed number vigor ($R^2_{adj} = 0.37$)				Seed number tolerance ($R^2_{adj} = 0.96$)			
Trait	Estimate	S.E.	*P*	BIC	Estimate	S.E.	*P*	BIC
Height CCI	–	–	N.E.[a]		**−0.87**	0.092	0.0025	29.41
Height TLA	–	–	N.E.		**0.87**	0.094	0.0027	30.16
Height NL	–	–	N.E.		**–**	–	N.S.[b]	0.47
CCI plasticity	–	–	N.E.		**2.00**	0.172	0.0014	28.91
TLA plasticity	–	–	N.E.		**−1.02**	0.133	0.0047	29.21
NL plasticity	**0.66**	0.265	0.0369	28.45	**−0.93**	0.113	0.0037	28.33

Significant linear selection gradients (estimate) with associated standard error (S.E.) and *P*-values (*P*) are presented in bold type

Note: To test whether trait mean value and plasticity of growth components in response to defoliation affected (1) the average fitness (vigor) or (2) the fitness plasticity of a given half-sib family (j), I used a stepwise regression analysis (Strauss et al. 2003; Wise et al. 2008) to control for correlations between independent variables. The Bayesian information criterion (BIC) was used, introducing a penalty term for the number of parameters in the overall models (†), to find the traits that explain the higher variance in it. I regressed relative values of seed set grand mean ($\bar{W}_j$) (averaged over control and defoliated plants), and their plasticities (plW_j), over the estimates of trait means (i.e., elevation of the reaction norm, $\bar{X}_j$) and plasticities (i.e., steepness of the reaction norm, plX_j) for all traits (Camargo 2017):

(a) $\bar{W}_j = Constant + \alpha_0 \overline{CCI_j} + \alpha_1 \overline{TLA_j} + \alpha_2 \overline{NL_j} + \beta_0 plCCI_j + \beta_1 plTLA_j + \beta_2 plNL_j$

(b) $plW_j = Constant + \alpha_0 CCI_j + \alpha_1 TLA_j + \alpha_2 NL_j + \beta_0 plCCI_j + \beta_1 plTLA_j + \beta_2 plNL_j$

[a] N.E. indicates that the trait did not enter in the final model

[b] N.S. indicates that the trait has not a significant effect

The last statement implies a change in perspective for tolerance studies. In the "operational" definition of tolerance, selection "operates" at this level; and hence, the heritability of fitness is required if selection leads mean population fitness to increase over generations (i.e., Fisher 1930). But if by evolutionary change of tolerance, one means change in phenotypes (Lewontin 1970), rather than mean fitness, is the underlying trait reaction norm that must both be heritable and selected for (Schlichting and Pigliucci 1998; West-Eberhard 2005), not the fitness differences (Okasha 2006). Therefore, the patterns of tolerance that we see in the natural world are a "by-product" of the action of the underlying phenotypic plasticity and natural selection working upon it.

The Nature of Selection: An Often Tacit, but Paramount Discussion for Tolerance Studies

Is selection proceeding in the phenotypic values expressed in each of the environments of herbivore damage or the targets of selection are the slope and elevation of the underlying trait reaction norm? This question had raised one of the most heated

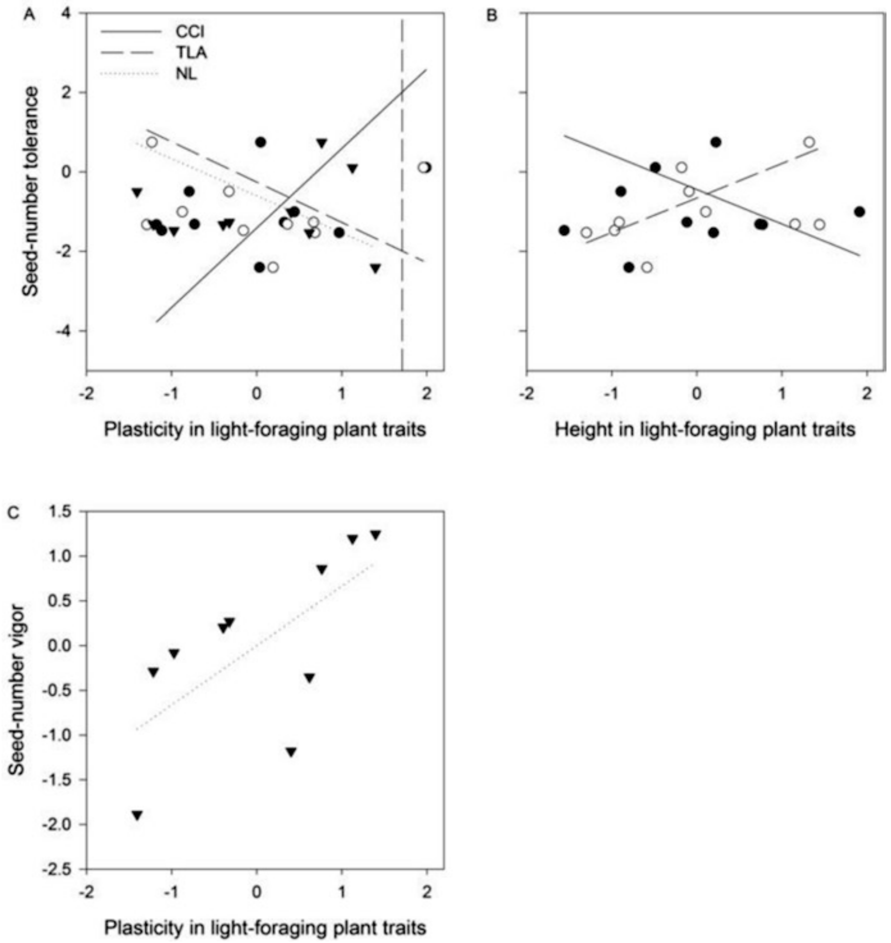

Fig. 4.E1 Significantly regression coefficients of plasticity and height of light-foraging traits versus the seed number tolerance or vigor in response to defoliation of ten genotypes of *Datura stramonium*, growing in a high-light environment. The slopes of the regression lines observed were obtained from separate regressions of the numbers of seeds (vigor or tolerance) on light-foraging traits, after controlling for trait correlations. The vertical reference line in the upper left panel indicates zero plasticity of unstandardized values only for the total leaf area; the other plasticities had positive values in response to defoliation. Plasticity is the difference of defoliated minus control plants. Symbols in all panels as in panel A

discussions in evolutionary biology (Via 1993 vs. Scheiner 1993b; for discussion, see Schlichting and Pigliucci 1998). The balance of the evidence is finally inclined to see natural selection as acting on the capacity for environmental responsiveness of the phenotype (West-Eberhard 2003; Pigliucci and Müller 2010), which is a combination of traits (i.e., an heritable phenotype, West-Eberhard 2003; Shipley et al. 2016) with a lifetime reproductive output of an individual possessing it (Shipley et al. 2016). It means that plasticity and the mean phenotype of a reaction norm per se are traits (West-Eberhard 2003). This “trait view” of a reaction norm is based on the observation that selection on variation in these traits can alter the degree of plasticity and the mean phenotype of a reaction norm (Scheiner and Lyman 1991).

One consequence of the above definition for tolerance studies is that some underlying traits are more tolerant components than others in a given abiotic environmental condition since the target of selection is the reaction norm to damage in this condition. Therefore, if the abiotic environment changes, the targets of selection on the reaction norms to damage change too (e.g., Camargo 2017). Understanding tolerance to herbivory becomes even more defiant because of the potent effects of abiotic conditions, which may modulate various tolerance components via the complexities of the growth process, in several, but often, contrasting ways. For instance, plants growing at high light have a reduced amount of leaf area per unit plant mass (Poorter et al. 2009). When light levels are higher than 25 mol m^{-2} day^{-1}, for some species, the growth rate is already not stimulated, but the rate of photosynthesis per unit leaf area is still increasing (Poorter and Van der Werf 1998). It leads to a situation in which plants express thicker leaves (low values of specific leaf area, SLA), better defended from herbivores (Poorter et al. 2013), but the primary adaptive function is not driving by herbivory. Those plants with thicker leaves, slow growth, but higher photosynthetic values can mitigate better the fitness effects when damaged by herbivory (Hilbert et al. 1981; Camargo et al. 2015). In other words, herbivores “recruit” these traits, and they become an aptation (i.e., any trait that currently contributes to fitness; Lloyd and Gould 2017) for tolerating it. Physiological interactions become even more complex when more environmental factors interact (reviewed in Poorter et al. 2013).

Therefore, there is no “Holy Grail” mechanism that we are looking at it. Tolerance is more a process for coordinating, managing, and distributing shared resources, in essence, a physiological one, that depends on the coordination of many environmental elicit traits. Seeing tolerance as a process implies a different ontology of the phenomenon. The focus of a process ontology is explaining stability or the appearance of stability in biological systems (Dupré 2018). Tolerance is mainly a homeostatic mechanism, which maintains the stability of the internal environment despite herbivory. Its function is to accommodate new environmental conditions acting immediately after damage to buffer the phenotype from its detrimental effects. Damage can induce phenotypic plasticity that initially might have both negative and positive effects on fitness (i.e., phenotypic accommodation; West-Eberhard 2003) (see Example 4.1), effects that we see in the patterns of tolerance. In this sense, there is the possibility that tolerance to herbivory is a *functional novelty* (West-Eberhard 2003), one that is likely to be viable and compatible (i.e., an aptation) with normal physiology (and ontology) of resource capture.

Preexistence plasticity for resource capture can be the source of this functional novelty. A novel aptation can coexist alongside an established adaptation for abiotic resource capture (see West-Eberhard 2003, p. 162). The new aptation would allow the individuals of a lineage to begin to exploit a different function (to cope with herbivory) without abandoning an established one. It is plausible to tolerate herbivory; the fitness reaction norm has been shown influence by the background imposed by fundamental growth rules since generic variation in intrinsic growth rate can lead to different tolerance patterns (e.g., Weis et al. 2000; Camargo et al. 2015; Camargo 2017). The relationship between a fitness reaction norm and the underlying phenotypic plasticity is by nature multivariate and depends on the underlying plasticity integration (i.e., the relationships between plastic responses of different underlying traits as correlations (Schlichting 1986; Schlichting and Pigliucci 1998, e.g., Camargo et al. 2015, Camargo 2017). West-Eberhard (2003) discussing preexistence plasticity asks (in her words).

Why Would an Individual Begin to Adopt a New, Possibly Clumsy Alternative When an Old, Relatively Refined One Is Available?

One of the benefits of the conservative (old) strategy is that populations would be *buffered* against decline (or extinction) as it acquires a new adaptation (West-Eberhard 1986, 2003, p. 162). In other words, individuals are gaining time to settle the arrival of the adaptation. Plasticity integration is the process at the center of this buffering effect, and it helps to the *phenotypic accommodation* of the environmental stress-induced variations employing adaptive mutual adjustments, without genetic change (West-Eberhard 2005). Hereafter, the author explains this idea a bit further and presents some of the reasons to be adopted by tolerance studies. Mainly, the author's focus is on viewing plasticity and the elevation (of a particular set of traits related to RGR, see below) of underlying reaction norms as traits per se, whose hierarchical integration is responsible for the variation observed in the fitness reaction norm to damage.

Plasticity Integration and the Identification of Tolerance Mechanisms

Some studies have examined the magnitude of heritable variation in growth-related traits (physiological and morphological) and the relationships between them and fitness (reviewed in Ackerly et al. 2000, e.g., Verhoeven et al. 2004; Jansen et al. 2019). Several of these ecophysiological characters are putative components of tolerance (reviewed in Rosenthal and Kotanen 1994; Strauss and Agrawal 1999; Stowe et al. 2000; Tiffin 2000; Fornoni et al. 2003). A particular set of traits have relative importance because they relate to resource capture, which is fundamental to tolerate herbivory (McNaughton 1983). These traits are the growth determinants (Hunt 1982). The variation in relative growth rate before and after damage can be quantified via three traits, using a growth analytical approach (Poorter and Van der Werf

1998; Poorter and Nagel 2000): first, the net assimilation rate (NAR, the increase in biomass per unit of time and leaf area), which correlates with the whole-plant net photosynthetic rate; second, specific leaf area (SLA, leaf area per unit of leaf biomass), which is a parameter that reflects aspects of leaf morphology such as leaf density and thickness; and third, the biomass allocation to leaves (LWR, leaf dry weight per total plant dry weight). Thus, RGR can break down (factorized) into these traits as follows: RGR = NAR × SLA × LWR. The product of SLA and LWR is the leaf area ratio (LAR, the ratio of leaf area per plant biomass). These growth determinants have been shown to influence plant tolerance, as the plant's active response to damage (Oesterheld and McNaughton 1988, 1991; Anten et al. 2003; Van Staalduinen and Anten 2005; Van Staalduinen et al. 2010; Dobarro et al. 2012; Camargo et al. 2015), and are directly involved in growth before damage (Poorter and Nagel 2000; Shipley 2006; Camargo et al. 2015).

Growth determinants can control the rate of growth/regrowth, increasing RGR before defoliation and buffering or increasing the differences of growth in the ontogenetic response to defoliation (Camargo et al. 2015). The contribution of these mechanisms to growth differs at interspecific (e.g., Van Staalduinen and Anten 2005) and intraspecific levels (e.g., Camargo et al. 2015) and between plants growing in different abiotic conditions (Coughenour et al. 1990; Oesterheld and McNaughton 1991; Van Staalduinen and Anten 2005; Van Staalduinen et al. 2010; Camargo 2017). Camargo et al. (2015) have shown that the contribution of growth determinants to regrowth ability changes throughout the ontogeny and is dependent on a complex interaction between these plastic responses to defoliation in the annual herb *Datura stramonium*. For instance, in early and late stages of the ontogenetic response to damage, the buffering (restoring the RGR of control plants by damaged plants) and overcompensation of growth (higher values in defoliated plants) are mainly achieved because plasticity in growth components did not have a trade-off. In the middle stages of the ontogeny, on the contrary, a trade-off between these plastic responses (i.e., NAR and LWR) was essential to maintain homeostasis (equal RGR values in control and defoliated plants) of the ontogenetic response to damage. NAR and LWR relate to compensatory growth of other herbs (Oesterheld and McNaughton 1988; Oesterheld and McNaughton 1991; Van Staalduinen and Anten 2005; Van Staalduinen et al. 2010). Additionally, Camargo (2017), using 36 genotypes from a hybrid population, showed that tolerance and vigor of seed number were related to the plasticity to defoliation of different growth components (that showed genetic variation) in *Datura stramonium* and that different nutrient environments change the contribution (i.e., as the trait identity or the strength of the correlation between trait values and fitness) of these traits to the fitness reaction norm. Other species had shown genetic variation for plasticity in RGR, and its growth components to defoliation (Jansen et al. 2019).

Very often, growth determinants vary to a greater extent than RGR because their primary role is buffering the differences in RGR due to differences in resource supply (Poorter and Nagel 2000; Shipley 2000; Züst et al. 2015). Plants in their natural environments do not achieve a growth rate close to their potential RGR due to different environmental stressors (Poorter 2002). Therefore, in the face of herbivore pressure, selection could not be for a high RGR that permits increases of tolerance. Alternatively, increases of tolerance may be due to a selection of one or more traits underlying RGR given the strong correlation between some growth determinants and RGR in damage environments, and in this way, growth determinants are good candidates to test hypotheses regarding the causes of variation of tolerance (e.g., Züst et al. 2015; Camargo et al. 2015; Camargo 2017; Salgado-Luarte and Gianoli 2017) and vigor (Camargo et al. 2015; Camargo 2017) to defoliation. Additionally, if growth determinants in response to herbivory change with the abiotic environment, then it would be the driving force behind tolerance and would set up the template for the type of fitness reaction norm that can evolve (Camargo 2017).

Growth determinants are part of a plethora of traits related to resource acquisition in plants (McKenna and Shipley 1999; Poorter et al. 2013), which favor the increase of total plant mass, and therefore are good candidates to be a part of a more comprehensive tolerance mechanism (Fig. 4.2). To decompose the underlying parameters of tolerance into increasingly more specific components (i.e., a mechanistic top-down model, Poorter et al. 2013) opens the possibility to examine which of the underlying traits vary between treatments, genotypes, or species and which ones would remain constant (Poorter et al. 2013). As a result, tolerance should be seen as a hierarchy of processes acting and integrated at different levels (molecular, physiological, and so forth). The hierarchy that stands in need for explanation should be the center of tolerance studies. The use of growth-related traits to the study of tolerance to herbivory is an example of the fusion of physiological ecology

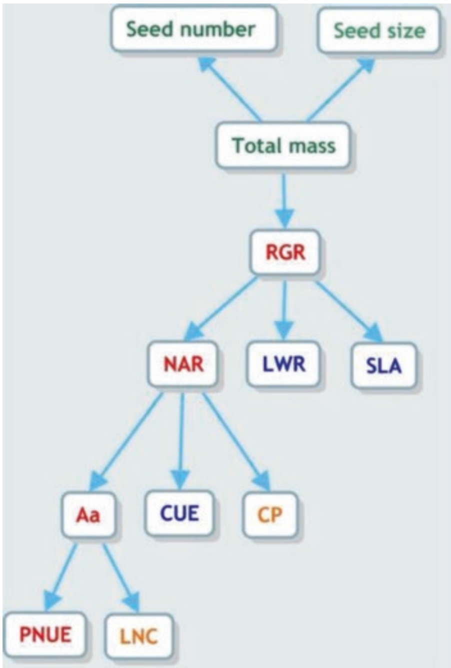

Fig. 4.2 A mechanistic top-down model of the total plant mass into some component variables. The traits have been reported as putative tolerance traits but stand in need to be tested as tolerance components and explored as a mechanism of tolerance (see Examples 4.1, 4.2, and 4.3 for details). In the model, fitness components (seed number, seed size) are conceived entirely dependent on the decomposition of total biomass. Trait font colors are in red for rates, blue for ratios, and orange for chemical or anatomical details. Abbreviations are listed in Table 4.1

and evolutionary biology that has been proven useful (Feder et al. 2000; Ackerly et al. 2000; Arntz and Delph 2001).

Plasticity integration of a tolerance mechanism can be studied using the tools of structural equation modeling and path analysis (Shipley 2016; Pigliucci and Kaplan 2006), which have the power to discover novel insights for tolerance mechanisms of our systems (Example 4.2). To accomplish this, one should use plasticity and elevation estimates of reaction norms to damage, rather than mean phenotypic estimates in each of the environments of damage used (e.g., Scheiner and Callahan 1999). Path analysis is a powerful tool to study the effect that the abiotic environmental variation has on the association of the fitness reaction norm vs. underlying traits reaction norms to damage (as long as one had estimated them in different abiotic environments). This approximation has been proved fruitful to the study of the action of selection in growth-related characters (Verhoeven et al. 2004), life-history traits (Scheiner and Callahan 1999), and the integration of life-history traits (Pigliucci and Kolodynska 2006). These examples show that path analysis is successful to estimate selection (Scheiner et al. 2000) and phenotypic integration (Pigliucci and Kolodynska 2006), as long as one uses genotypic-trait estimates and considers the changes in path coefficients among abiotic environments as a measurement of changes in integration. The studies of causal mechanisms of tolerance might gain a great benefit using this approach. The author has taken these two perspectives (multiple regression and path models) into account and proposed a method for the recognition of tolerance mechanisms as proper aptations to herbivory (Example 4.3).

Example 4.2: Tolerance Mechanisms and Structural Equation Modeling
Structural equation models (SEM) are designed to allow for the representation of scientific hypotheses as causal (probabilistic) networks (Laughlin and Grace 2019; Shipley 2016). It is this causal network that allows linkage to defense theory to represent explicitly causal structures (i.e., mechanisms of tolerance). For instance, by specifying an indirect effect in a causal network involving three traits, that is, the plasticities of net assimilation rate, total biomass, and seed number, it is possible to represent a tolerance mechanism within the model (Fig. 4.E2a). What is being hypothesized is that the plasticity of total biomass is the trait that conveys the effects of net assimilation rate on seed number. Using only the regression analysis, we have an explanation for the direct links in a model, but the explanation is not testable (Laughlin and Grace 2019). SEM permits to test that the causal network is not supported or incomplete using conditional independence (Shipley 2016).

When a particular model is unsupported by the data, we conclude that there is no support for the proposed mediation mechanism (Laughlin and Grace 2019). If incomplete, we conclude that there is an additional mechanism whereby the net assimilation rate influences seed number using, for instance, an additional pathway not mediated through total biomass (Fig. 4.E2.b). This latter finding is when we discover a new tolerance mechanism operating that was unsuspected and which is only revealed when the network contains mediation hypotheses (Laughlin and Grace 2019). This kind of inference is hard to achieve using only regression analysis to identify tolerance components (cf. Example 4.1). Other mechanisms can also be

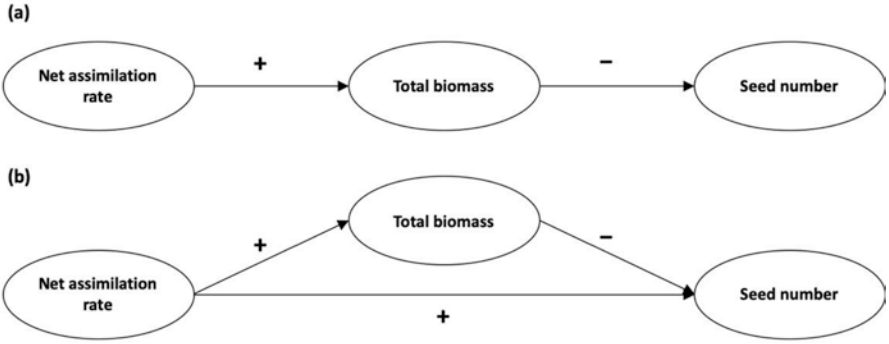

Fig. 4.E2 Indirect (**a**) and offsetting (**b**) effects that can lead to novel insights in tolerance mechanism research. The effects are illustrated using three trait plasticities: net assimilation rate, total biomass, and seed number. The signs in (**a**) and (**b**) are hypothetical. However, in (**b**) net assimilation rate is linked to seed number by two different pathways that have net opposing signs (after multiplying the coefficients – signs – along the length of the path; Laughlin and Grace 2019). It means that the positive direct effect of the net assimilation rate on seed number offsets the negative indirect effect of net assimilation rate on seed number mediated through total biomass

revealed through the use of SEM (see thoughtful reviews in Laughlin and Grace 2019; Shipley 2016).

Example 4.3: A Method to Identify Tolerance Mechanisms
What stands in need for explanation in tolerance mechanisms is the hierarchical structure of causal patterns between underlying reaction norms and the resulting fitness reaction norm, not just the statistical associations. The intention is not to replace selection gradients or traditional path analysis but make these complementary. The main goal is testing causal hypotheses about the action of selection. It can be accomplished in two steps. First, we need to identify tolerance components (see Example 4.1 for how to run it), and second, what are the structures of tolerance mechanisms. Regression analysis is then a tool to provide the initial steps (a statistical summary) from which to branch to a more sophisticated statistical approach to disentangle the hierarchical interaction of different components of tolerance (e.g., Byers 2005; Pigliucci and Kaplan 2006).

Identifying Tolerance Mechanisms

- If one has not a particular set of the hypothesis at the start of the experiment to construct a path model, then one can use exploratory path analysis (Shipley 2016). This approach permits the estimation of potential path models of the plants. However, the functional value of the model must be evaluated using some empirical knowledge of the system (e.g., Shipley 2004).
- Avoid mixing the ontogenetic determination of traits (Scheiner and Callahan 1999; Scheiner et al. 2000). Traits determined earlier in the life cycle cannot be influenced by traits determined later (Byers 2005).
- Use signed plasticity values for height and slope of tolerance component reaction norms and tolerance and vigor of the fitness reaction norm.

- The X^2 statistic determines if the model fits the data, and if so, the probability value for this test would be higher than 0.05. However, there is a variety of criteria to evaluate model performance (Shipley 2016, e.g., Pigliucci and Kolodynska 2006). It is considered a tolerance mechanism.
- Once the tolerance mechanism has been specified, it can be fit to determine the partial regression coefficients between traits (Shipley 2016). The same causal structure can be fit in different abiotic environments.
- The strength and number of significant path coefficients reflect the amount of integration in the tolerance mechanisms. It is expected an increase of both the strength and number of significant path coefficients under more stressing conditions (Schlichting and Pigliucci 1998; Pigliucci and Kolodynska 2006).

To the author's knowledge, only one study tried to investigate the tolerance to leaf damage in the framework of phenotypic plasticity using path analysis (Juenger et al. 2000). However, in this study, there was used phenotypic estimates of the node numbers in the clipped and unclipped environment (following the method by Scheiner and Callahan 1999), and not the plasticity of the character per se. Besides, as the authors acknowledged it, the use of small family sample size, maternal lines, and the absence of other characters genetically correlated with node production no restrict the heuristic value of the study but its scope (Juenger et al. 2000). Notwithstanding, an essential caveat in selection analysis is the inclusion of important genetically correlated traits (Rausher 1992). It is essential for the identification of aptations to herbivory since tolerance is the result of the combined action of several traits (Wise et al. 2008), and more critical is the hypothesized hierarchical interaction (i.e., a tolerance mechanism) of different tolerance components that we need to test. Of course, some species could have specific tolerance mechanisms, and these traits (as the involved in life history) can be added to a path model (see fruitful modeling using abiotic environments in Verhoeven et al. 2004).

Up to date, no study has used path analysis to study the plastic integration of growth determinants in response to herbivory and different abiotic environments. Research in growth determinants in response to herbivory is an area that needs more attention because they are liable to measure in all plant species and encompass several physiological plant processes in a unique tolerance mechanism.

The Recognition of Tolerance Mechanisms as Proper Aptations to Herbivory

A proper aptation to herbivory should have what Vermeij (1987) in his theory of adaptation state as a clear distinction between different selective agents (in his words): "if the aptation is to become established, its benefits must outweigh any disadvantage that the trait has with respect to other environmental challenges." One can compare if the models derived from the data of plasticity to damage fit the data from another abiotic environmental condition that one wants to test (Byers 2005;

Pigliucci and Kolodynska 2006). The selection of underlying plasticity to damage (and the resulting fitness reaction norm) can be different depending on different abiotic environments. The action of selection on a tolerance mechanism can be different by each altering the pattern of plasticity integration (i.e., the magnitude and the number of significant path coefficients, e.g., approach by Pigliucci and Kolodynska 2006) or recruiting more traits (i.e., the causal structure of exploratory models change with the environment, e.g., Byers 2005). If selection can act at the integration level (e.g., Ordano et al. 2008), a whole mechanism of tolerance has an adaptive value.

The two approaches mentioned above should be used with two caveats. First, a tolerance mechanism to damage, in a particular species, should be maintained by selection throughout a given abiotic environmental gradient (e.g., a light gradient) to accept what Vermeij established as a condition for an aptation (a functional one in this case). Then, the last view supposes that an environmental discrimination process (i.e., a gradient of a given abiotic condition) may select based on a particular (a unique tolerance mechanism) set of traits interacting with it (Pigliucci and Kaplan 2006). Therefore, our best approach is the hypothesis testing of a particular path structure across different levels of the abiotic resource (Pigliucci and Kolodynska 2006).

Second, if one is trying to look for how natural selection is recruiting new traits for a tolerance mechanism depending on different abiotic conditions (i.e., said light vs. water availability), in contrast to a gradient of a given one, the best option is to compare models with different structures using confirmatory path analysis (Shipley 2009). Tolerance mechanisms change over time and are part of an environmental matrix that is inherently hierarchical or otherwise multilevel; that is, we often take measurements on the same individuals for ontogenetic traits (like RGR), they are nested in different populations and species that pertain to different geographical areas, and so on. These traits measured at different hierarchical levels can be explored and tested with the mechanics of generalized mixed model regression using "generalized multilevel path models"[2] (Shipley 2009; Shipley 2016, e.g., Thomas et al. 2007).

The recognition of the difference between a putative tolerance trait, a tolerance component, and a mechanism of tolerance is a first step for the proper study of the underlying causal nature of tolerance. Each of these concepts requires a methodological approach to get its identification. The recognition of the multivariate nature of tolerance mechanisms (and the proper use of a methodological reductionism), in which the understanding of the parts of a system can be seen not as a set of interlocking traits but as a hierarchy of them acting at different levels, is the best way to digging deep into the causal nature of tolerance. It is this causal nature whose physiology is mainly concerned with several internal processes that allow organisms to

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[2] An in-depth account of the literature devoted to path analysis and SEM is, of course, beyond the scope of this chapter. For a good summary of the best techniques available and its applications on the software R, see Shipley et al. (2016).

survive and maintain its thermodynamic disequilibrium with its multidimensional environment.

Eco-Evolutionary Dynamics Mediated by Mechanisms of Tolerance to Herbivory

The understanding of tolerance mechanisms and the recognition as genuine aptations to herbivory can throw so much light about one of the fundamental questions in plant-herbivore interactions. Can tolerance affect plant-herbivore coevolution by imposing selection on herbivores? (Stinchcombe 2002) Plant regrowth (and all of the traits that control it, i.e., growth determinants) is the fundamental trait that had received considerable attention to answer this question in the literature. For instance, in the willow system, the enhanced new leaf production because of willow regrowth produces an intraindividual plant variation of leaves quality that can impact the fitness of the herbivore (Utsumi et al. 2009, cf. Garrido and Fornoni 2006). The leaves of the regrowth shoots have more significant water and nitrogen content than those of non-regrowth shoots (Utsumi and Ohgushi 2007, Utsumi et al. 2009). It leads to the evolutionary development of a particular feeding preference for young age leaves in adults of the leaf beetle *Plagiodera versicolora* (Utsumi et al. 2009). This feeding preference is a heritable quantitative trait that has intraspecific variation according to differences in the local intensity of induced plant regrowth (Utsumi et al. 2009). The populations with higher induced plant regrowth display a stronger feeding preference for immature, new leaves by adult herbivores, which almost exclusively forage on new leaves of willows in the field. Other populations with lower induced regrowth show a weak preference for new leaves by adult herbivores, which non-preferentially feed on new and mature leaves (Utsumi et al. 2009). Plant regrowth could have community-level consequences too. An increased overall abundance and species richness of herbivorous insects and predaceous arthropods and species composition differing between regrowth and not regrowth shoots have been reported more often (e.g., Utsumi and Ohgushi 2009; reviewed in Utsumi 2013).

Increased levels of plant regrowth could explain why tolerance can show a phenotypic escalation (e.g., Agrawal and Fishbein 2008). It has been predicted a phenotypic escalation in defense trait expression as plant lineages diversify, resulting in novel traits, which evolution is incremental and directional (Ehrlich and Raven 1964; Farrell and Mitter 1998; Vermeij 1994; Jablonski 2008). The strategy of defense that most have appealed to accomplish this particular case of "escape and radiate" hypothesis (Ehrlich and Raven 1964) is resistance. However, recently it was founded a macroevolutionary escalating regrowth ability, in contrast to declining resistance in *Asclepias* species (cf. the pattern shown by phenolics in Agrawal et al. 2009). Tolerance might be favored over resistant traits during the diversification process, especially when plants are primarily consumed by specialist herbivores (Agrawal and Fishbein 2008).

These results are congruent with the finding that some invasive species are not escaping but tolerating herbivory in the new range; they are persisting despite high rates of herbivore damage in the field (Del-Val and Crawley 2005; Ashton and Lerdau 2008; Fornoni 2011). It suggests that tolerance may also play a significant role in enhancing the ecological breadth of species and add evidence in favor of major levels of damage favoring tolerance in natural populations (Stowe et al. 2000). The discovery of more tolerant components to identify a more comprehensive mechanism of tolerance that permits across species comparisons in a phylogeny (see Agrawal 2007 for a review of comparative methods) would help to explain the evolution of increased tolerance.

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Chapter 5
Natural Selection of Plant Defense Against Herbivores in Native and Non-native Ranges

Juan Núñez-Farfán and Pedro Luis Valverde

Abstract Natural enemy release is one main historical event that promotes the evolution of alien-colonizing plants in a new range. During this process, it is expected that selection on characters that mediated the interaction between plants and their herbivores in the native range will change the average trait values in the new range and other correlated responses of introduced populations where the herbivores are absent. However, few studies have measured natural selection on plant defense in invasive species in the introduced range despite this assumption. We present a summary of the evidence available about the changes in characters associated with resistance, including chemical and physical traits, and tolerance to damage, between plant populations in native and non-native ranges. We present the basic predictions of selection on defense and the measurement of selection in the few studies carried out so far. We stress on the study of evolutionary ecology of plant-herbivore interactions in new environments, by means of quantitative genetics and the analysis of natural selection.

Keywords Generalist herbivores · Invasive plant species · Natural selection · Plant defense · Quantitative genetics · Resistance · Tolerance

Local adaptation of populations is a common outcome of evolution (Schluter 2000; Leimu and Fischer 2008). This implies that local individuals in their home habitat have higher fitness than foreigners (Schluter 2000; Kawecki and Ebert 2004). The theory behind the evolution of local adaptation, one of the most common

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phenomena produced by evolution on Earth, constitutes the main evolutionary framework to study the evolution of invasive species (reviewed by Colautti et al. 2004; Dlugosch and Parker 2008; Bock et al. 2015; Colautti and Lau 2015; Odour et al. 2016; Hodgins et al. 2018).

Darwin (1859, Chap. IV) clearly foresighted this phenomenon, by recognizing the relative nature of adaptation and that, in most habitats, native species could be replaced by introduced ones:

> "No country can be named in which all native inhabitants are now adapted perfectly to each other and to the physical conditions under which they live; that none of them could anyhow be improved; for in all countries the native have been so far conquered by naturalised productions, that they have allowed foreigners to take possession of the land. And as foreigners have thus everywhere beaten some of the natives, we may safely conclude that natives have been modified with advantages, so as to have better resisted such intruders."

Nowadays, among others, anthropic disturbance of natural ecosystems (i.e., land-use change) has permitted the establishment of alien species (Sax and Brown 2000; Hierro et al. 2005; Moles et al. 2008). Land-use change and biotic exchange count among the first and fourth, respectively, more important drivers of biodiversity change of future scenarios on Earth (Sala et al. 2000). Whether an alien species becomes invasive in a new range obeys to different factors and the characteristics of species that ultimately would determine its local abundance, range distribution, and habitat generality, which are likely to be associated with its competitive ability, dispersal ability, and environmental tolerance, respectively (van Kleunen et al. 2018).

The successful establishment of a non-native species may give rise to new eco-evolutionary dynamics at the population, community, and ecosystems levels (Hendry et al. 2011). New biotic interactions would be established potentially affecting trait distribution of interacting species. At the same time, the alien species lost the interactions with species of its former, native, range. Under such scenario, a new evolutionary history begins in the new range.

A plant species that colonizes a new range may be free from factors that regulated population dynamics in its native range (Crawley 1987; Keane and Crawley 2002). Considering biotic factors, the interaction with predators, pathogens, competitors, and mutualists of its former habitat would be absent. We assume that such biotic interactions affect individual fitness in the native habitat. Actually, the evolutionary theory provides the basis to comprehend the evolution of invasive species in a new habitat (Hendry et al. 2011) in comparison to its conspecifics, ideally the population of origin, in the native habitat.

Resistance and Tolerance to Damage by Herbivores

Plant defense against natural enemies (herbivores, pathogens) includes two complex characters named resistance and tolerance (Núñez-Farfán et al. 2007). Resistance is the ability of a genotype to prevent or to keep at low level the damage inflicted by its natural enemies. Plant resistance is provided by different traits that impede or reduce the access to living tissues of plants. Such traits encompass physical characters like spines (Cooper and Ginnett 1998) or leaf trichomes (Levin, 1973; Valverde et al. 2001), as well as chemical compounds, like digestibility reducers (e.g., tannins, Barbehenn and Constabel 2011; latex, Konno 2011) or toxins (e.g., alkaloids, Mithöfer and Boland 2012; glucosinolates, Merillon and Ramawat 2017), that deter herbivores or affect their performance. Thus, the traits that protect plants from the attack of herbivores and their relative importance in plant resistance vary among plant species and populations (Thompson 1994; Muola et al. 2010; Castillo et al. 2014).

Tolerance, on the other hand, is the ability of a genotype to attenuate the fitness costs to herbivores (Núñez-Farfán et al. 2007). Because tolerance is a property of a genotype, tolerance can be viewed as a reaction norm of fitness in a varying gradient of damage (Simms 2000; Fornoni et al. 2003). Like plant resistance, the plant traits linked to the tolerance response are numerous and vary between plant species with different life history and life forms (see Stowe et al. 2000). Because of the multicharacter nature of plant resistance (or tolerance), it is safe to affirm that the variance of single plant defense trait, either physical, chemical, or functional, explains only a fraction of the variance in resistance (or tolerance) in a population. For the very same reason, genetic variance in tolerance and/or resistance may represent variance in different traits in different populations, owing to the type and pressure exerted by enemies. Thus, selection of any strategy, resistance or tolerance, may favor, potentially, genetic correlations between traits. This is relevant when analyzing the changes produced on tolerance and resistance in the absence of a selective agent (i.e., natural enemies).

Simms and Triplett (1994) advanced that resistance and tolerance may function as redundant strategies to cope with natural enemies (i.e., pathogens) and, if costly, may constitute a trade-off. When the plants' natural enemies are present, natural selection will favor either the more tolerant or the more resistant genotypes if both defense mechanisms are costly in terms of fitness (Mauricio et al. 1997). Thus, this predicts a negative genetic correlation between resistance and tolerance. However, the differences in the fitness costs and benefits of each strategy, may allow the evolution of mixed strategies (i.e., genotypes that simultaneously allocate resources to both strategies (Fornoni et al. 2004; Núñez-Farfán et al. 2007)). Núñez-Farfán et al. (2007) revised the hypotheses that analyze the potential for mixed strategies of plant defense.

Hypotheses of Plant Invasion in Relation to Enemy Release

Analyzing the alien plants introduced in the British islands and the cases of insects introduced for biological control of weeds (e.g., *Lantana* and *Opuntia*), Crawley (1987) found that the importance of herbivore release, as a cause of successful establishment of plants, was suggestive but not conclusive. On the other hand, in the case of insects released as biological control, it was assumed, in that time, that insects kept at low densities in their native habitat would be more successful to establish as control agents when released from their predators and parasites in the new habitat. The assumption here is that these insects had low densities because they were strongly biotically regulated in their native range. The evidence indicated that insect species more abundant and widespread in their native habitats were more likely to establish than species that occur at low densities and have patchy distributions. Yet, “it would be wrong to suggest that release from natural enemies is irrelevant for the rapid spread of biocontrol agents” (Crawley 1987).

Enemy Release and the Evolution of Increased Competitive Ability Hypotheses of Alien Plants

Let’s suppose that in a native range a plant species has been, historically, under selection by its natural enemies to change defense characters. In the long term, other things being equal, selection will produce a phenotypic distribution of defense traits close to the optimum value of defense against enemies, considering ecological and evolutionary constraints. Obviously, the distribution of trait values of defense translates, at least partially, into individual fitness differences. However, in a new range where enemies are absent, the function of fitness in relation to such defense traits is expected to change (Franks et al. 2008; Valverde et al. 2015; Castillo et al. 2019).

The enemy release hypothesis (ERH) states “that plant species, on introduction to an exotic region, should experience a decrease in regulation by herbivores and other natural enemies, resulting in an increase in distribution and abundance” (Keane and Crawley 2002). This condition opens the opportunity for evolution not only of the alien plants in the new environment but also for native plants and herbivores. Generalist herbivores may use a new host plant, and native plants must compete with a new member of the community (Keane and Crawley 2002). What would be the “route” of evolution? Considering the alien plant population, the enemy release renders investment in defense useless and, through time, genotypes with high investment in defense will be selected against, while those genotypes investing in vegetative growth or reproductive effort will be favored (Blossey and Nötzold 1995). Populations released from pest pressure in a new environment may, in consequence, allocate resources to fitness-enhancing traits that in their native environment were invested in avoiding, resisting, or diminishing damage by natural enemies (i.e., to resist or to tolerate damage imposed by enemies). Based on optimal defense theory (Coley et al. 1985; Herms and Mattson 1992), this condition may favor the reallocation of such resources to increase plants’ competitive ability in the new environment (i.e., biomass allocation; Blossey and Nötzold 1995). This hypothesis has been termed the evolution of increased competitive ability (EICA; Blossey and Nötzold 1995).

Different hypotheses and predictions have been derived from this enemy release event, for instance, assuming that alien plants in the new range evolve toward a reduction in the expenditure of resources to defense may change to invest on "cheaper defenses" (i.e., qualitative instead of quantitative). This shift in their strategy toward qualitative, low-cost, chemical defense would be a response to function against generalist herbivores in the new habitat (Shift in defense hypothesis, SDH; Müller-Schärer et al. 2004; Joshi and Vrieling 2005; Doorduin and Vrieling 2011), where generalist herbivores are the more likely to attack alien plants (contrary to specialist herbivores of native plants in the new range; Keane and Crawley 2002). This shift in defense predicts a divergence in investment in qualitative instead of quantitative defense between the alien plants in the introduced range and its conspecifics in its native, home, habitat (Joshi and Vrieling 2005). Thus, in the introduced range, a reduction and increase in defense against specialist and generalist herbivores are expected, respectively (Doorduin and Vrieling 2011). Consequently, such qualitative plant defenses might constitute evolutionary innovations, "novel weaponry," to mediate the interaction with novel local herbivores that have not co-evolved with the alien plants, as posit by the novel weapons defense hypothesis (NWH; Callaway and Ridenour 2004).

A fair amount of reviews, meta-analyses, have contrasted the empirical evidence against the predictions derived from each hypothesis. However, it is not the main purpose of this chapter to make an exhaustive analysis of each study, but we offer a summary of the important results, in the light of the main hypotheses, derived from one recent meta-analysis performed by Zhang et al. (2018). This analysis includes data on defense-related traits (i.e., resistance and tolerance) in the introduced and native ranges of invasive species, with at least data of two populations in both introduced and native range of alien species. Second, such studies provide information on the type of herbivore (specialist or generalist). Third, an examination of the papers helped to determine the origin of collection of herbivores, whether it was from the native range (and co-occur with the native populations of the invasive species) or from the non-native range (thus co-occur with the invasive plants). Tolerance was measured as biomass, growth rate, or reproduction. All studies were conducted in a common garden.

Zhang et al. (2018) tested if introduced populations show reduced defense (including defense characters, damage received, and tolerance to damage (Table 5.1)), against specialist herbivores but increased against generalist herbivores, in relation to native populations. Their results are in line with predictions of the SDH (Table 5.1). First, they detected that while no differences between introduced and natives were found for physical defenses and toxins (qualitative defense), a significant reduction for quantitative defense (digestibility – reducers, like tannins) was detected in the invasive populations. Further, introduced populations had lower resistance than native populations against specialist herbivores collected in the native range, but higher resistance to generalist herbivores collected from the non-native range. Finally, introduced populations display higher tolerance to damage by generalist herbivores than native populations. Increased tolerance and resistance to damage by generalist herbivores imply that both strategies covary (Zhang et al. 2018). Overall, the evidence indicates that invasive plant species continue allocating resources to resistance and that this allocation may be shifted toward

Table 5.1 Mean effect size of differences in defense between introduced and native populations of invasive plants

Defense category		Measure	No. of effect sizes	Mean effect size[a]	Result
Type of defense	Type of herbivore				
Resistance traits	–	Physical defense	14	0.025^{NS}	Introduced = native
Resistance traits	–	Digestibility reducers	9	$-0.264*$	Introduced < native
Resistance traits	–	Toxins	20	0.098^{NS}	Introduced = native
Resistance effects	Specialist	Damage by herbivores	14	$-0.254*$	Introduced > native
Resistance effects	Specialist	Performance of herbivores	21	-0.027^{NS}	Introduced = native
Resistance effects	Generalist	Damage by herbivores	20	$0.324**$	Introduced > native
Resistance effects	Generalist	Performance of herbivores	17	-0.021^{NS}	Introduced = native
Tolerance	Specialist	–	15	-0.016^{NS}	Introduced = native
Tolerance	Generalist	–	16	$0.108*$	Introduced > native
Tolerance	Simulated herbivory	–	13	-0.07^{NS}	Introduced = native

Modified from Zhang et al. (2018)

[a] Positive mean effect sizes imply that introduced populations have higher levels of defenses than the native populations. $^{*}p < 0.05$, $^{**}p < 0.01$, *NS* non-significant

other types of defense like tolerance to damage and suggest, following the hypotheses, that such changes might be adaptive if are the result of the interaction with novel herbivores in the new range. At any rate, we want to stress the necessity of study the evolution of plant defense to herbivores in invasive plants in their native and non-native habitats by mean experimental and field studies.

Evolution of Plant Defense of the Invasive Plants

Although the synthesis of empirical evidence strongly suggests the evolution of plant defense in the absence of herbivores in the introduced ranges (see Müller 2018, Zhang et al. 2018), we still need to determine whether natural selection is the process responsible for such an evolution. According to EICA, in the absence of herbivores, natural selection will favor genotypes with improved competitive abilities and reduced resource allocation to herbivore defense (Blossey and Nötzold 1995). This is the central assumption of most hypotheses (Franks et al. 2008; Fornoni 2011; Colautti and Lau 2015; van Kleunen et al. 2018; Castillo et al. 2019).

Some predictions, albeit simple, for the evolution of plant defense in invasive populations and the potential roles of natural selection are:

1.0 Lower damage by specialist herbivores on non-natives than on natives (ERH). Whether damage is equal between native and non-native populations, then this would imply either:

  1.1 Lack of defense evolution in the non-native range
  1.2 Damage to introduced populations by generalist herbivores or
  1.3 Damage by specialist herbivores from the introduced range or immigrant herbivores of the former native range

2.0 Following 1.0, selection against plant defense traits would be expected in the non-native populations in the introduced range (lower investment in plant defense; EICA).
3.0 Selection to increased competitive ability in the invasive range. Blossey and Nötzold (1995) suggest that competitive ability can be maximized by increasing vegetative growth or reproductive effort; EICA] and, thus, should be selected for:

  3.1 Other factors being equal, this predicts that non-native plants of a species will be selected to produce more biomass than individuals taken from the species in the native range (EICA).
  3.2 Specialized herbivores will attain higher performance on plants from the introduced range than from the native range (due to 2.0) (EICA); selection for tolerance is expected (see 5.2).

4.0 When alien plants in the introduced range are eaten by generalist herbivores (1.2), this might select for defense of low cost and against costly defenses (physical and digestibility reduces) (SDH).
5.0 Selection for tolerance to herbivory might be a ubiquitous response in different conditions.

  5.1 If tolerance is an ancestral condition of alien plants in the introduced range, costly, and damage in this new environment nil (1.0), then tolerance would be selected against since the benefits of tolerance are "cashed," paradoxically, when damage occurs.
  5.2 It seems likely that tolerance to herbivory will be selected for, once local herbivores from the introduced range include the invasive species as part of their host plants (see 1.2, 1.3).
  5.3 Tolerance might be selected for if it contributes to increase competitive ability of alien plants in the new range (3.1), if both tolerance and competitive ability shared traits (i.e., ability to regrowth), and are consumed by herbivores (e.g., 3.2), genetic correlations among traits are expected.

From these selective scenarios, it is clear that some responses would not be mutually exclusive and that some combinations are possible making more complex to extract crystal clear generalizations from empirical evidence. For instance, consider the following combination: [1.3] [3.1] [4.0] [5.2]. Under this situation, if linked to fitness, natural selection would act on to increase plant defense to herbivores, competitive ability and tolerance to damage of alien plants in the introduced range. Thus, if the conditions necessary and sufficient for natural selection are met (Endler 1987), we expect direct selection on the three characters (or its components). Yet, natural selection may favor combinations of defense traits, for instance, tolerance and chemical defense, because both offer fitness advantages in the light of generalist and specialist herbivores. Thus, the hypothetical situation outlined above must include a term that includes correlational selection (cf. Lande and Arnold 1983). Note, however, that to date few studies have measured natural selection to herbivores in invasive populations in the light of animal release (see below; Franks et al. 2004).

Natural Selection of Alien Plant Species When Herbivores Are Absent

Local Adaptation

The evidence of the commonness of local adaptation is at odds with the apparent success of alien plants (Sax and Brown 2000). First, in 76% of the studies in plants the local population outperforms the foreign one (Leimu and Fischer 2008). Second, colonization/invasion of new habitat might involve a small population size in many instances (Dlugosch et al. 2015). Third, population size is one of the best predictors of local adaptation in plants (Leimu and Fischer 2008). Thus, this means that an exceedingly large fraction of colonization events fails to success. Unfortunately, extinction in the new habitat of colonizers leaves no “fossils.” On the other hand, strong selection on alien plants, in the new habitat, may favor successful establishments and ultimately adaptive divergence. However, for evolutionary change, this makes necessary variation in plant populations both phenotypic and genetic (Bossdorf et al. 2005; Dlugosch et al. 2015). Further, environmental conditions in the new environment should impose strong and contrasting selective pressures on invaders (Franks et al. 2008, 2012; Valverde et al. 2015; Castillo et al. 2019). It is thought that habitat disturbance, nowadays disturbance by humans, facilitates success of invasive species.

Conditions for Natural Selection

Phenotypic variation of plant traits (i.e., defense) that mediate their interaction with plant enemies or that provide competitive advantages of alien plants in an introduced new habitat must be related to individual fitness (i.e., covariate) (Erb 2018) and be the result, at least partially, of the additive effects of genes involved in their phenotypic expression (Endler 1987; Falconer and Mackay 1996). Phenotypic variation is common, as its observation and measurement have given rise to the hypotheses (e.g., Darwin 1859; Elton 1958; Blossey and Nötzold 1995; Keane and Crawley 2002). However, whether genetic effects and their interaction underlay phenotypic variation and its relation to individual fitness is less well known. The evolution of defense against herbivores requires these conditions to be met.

A thorough review of the literature (Bossdorf et al. 2005; see above) aimed to answer these questions revealed that (1) phenotypic variation in defense and competitive ability among alien plant species is common, partially supporting EICA (direction of the differences between alien and native populations); (2) for molecular markers, adaptively neutral, native and non-native plant populations possess about the same amount of genetic variation, notwithstanding colonization is considered to be associated with small effective populations sizes; (3) quantitative data suggest genetic variance of phenotypic traits since experiment were run in common environments (greenhouse or common garden). Thus (4) this genetic divergence between alien and native population suggest that evolution could be the result of selection and/or drift (Bossdorf et al. 2005). However, a more recent meta-analysis (Uller and Leimu 2011) of genetic diversity in neutral molecular markers (i.e., microsatellite loci) found that:

1. Introduced plant populations had higher genetic variation than native populations, in contrast to insects and mammals where introduced populations had lower genetic variation.
2. Populations funded by individuals from a single source tended to have reduced genetic variation compared with native populations, whereas populations funded by individuals from different sources have higher genetic variation than source populations.
3. Genetic variation tended to be lost as the number of generations since the introduction increased, relative to the source or native population.
4. More genetic variation was lost when propagule pressure is low.
5. Highly invasive species have on average the same genetic diversity than less invasive species; and there is no evidence that the more invasive are the result of multiple introductions. Thus, data suggest that invasiveness is not limited by genetic bottlenecks (Uller and Leimu 2011).

To what extent genetic diversity represents genetic variance at quantitative characters? The evidence indicates that both measurements of genetic variation are only weakly correlated. Thus, quantitative genetic variation should be assessed if the interest of study centers on selection and adaptive evolution (Reed and Frankham 2001).

The methodology of quantitative genetics and natural selection can be used to determine the genetic variance of phenotypic traits between native and non-native populations and to measure natural selection in action, on traits predicted to change in the absence of plants' natural enemies (Franks et al. 2008; see above). This approach is a logic step in the study of phenotypic evolution of invasiveness (see O'Neil 1997; Murren et al. 2009; O'Donnell and Pigliucci 2010; Colautti and Lau

2015; Schueller 2007; van Kleunen et al. 2018; Sekor and Franks 2018a) but rarely applied to in the context of enemy release hypothesis (cf. Franks et al. 2004, 2008, 2012).

To accomplish this, we need to evaluate plant defense, fitness, competitive ability, etc., under a reciprocal transplant experiment between native and introduced ranges of relatives, in order to determine the amount of additive genetic variance of traits (Falconer and Mackay 1996; Schluter 2000; Kawecki and Ebert 2004). Different groups of relatives can be grown in order to "extract" the fraction of additive genetic variance (V_A) relative to total phenotypic trait variance (V_P) or heritability ($h^2 = V_A/V_P$). The groups of relatives to be assessed include parent-offspring, full-sib families, half-sib families, etc. (see Lawrence 1984). The average covariance between relatives in a phenotypic trait is proportional to the amount of additive genetic variance of the traits (see Lawrence 1984, Falconer and Mackay 1996). More importantly, the breeding value or additive genotype is twice the deviation of the average family value, of a given phenotypic trait, from the population mean of the trait (Falconer and Mackay 1996).

Selection analysis (Lande and Arnold 1983) can be executed on phenotypic values (individual plants) or on breeding values (families) (Simms and Rausher 1989; Rausher 1992; Núñez-Farfán and Dirzo 1994). Selection analysis on breeding values is preferred as it indicates the effect of selection on the additive genetic variance of a trait and prevents the effect of environmental correlations among traits (Rausher and Simms 1989). The linear selection gradients (β_i) and quadratic and correlational selection gradients (γ_{ii}, γ_{ij}) (see Lande and Arnold 1983) are estimated by multiple regression analysis; for linear (directional) selection:

$$w_i = \alpha + \beta_i z_i + \beta_j z_j + e \tag{5.1}$$

and for non-linear (stabilizing/disruptive) quadratic selection:

$$w_i = \alpha + \beta_i z_i + \beta_j z_j + \gamma_{ii} z_{ii}^2 + + \gamma_{jj} z_{jj}^2 + \gamma_{ij} z_i z_j + e \tag{5.2}$$

where w_i is relative fitness, α is a constant, z_i and z_j are standardized traits, and e is the error term.

The selection differential, S_i, of a trait (z_i) is the covariance (Cov) of such a trait with fitness [Cov (z_i, w)] and includes the direct effect of selection on plus indirect effects through the covariance with other traits (Lande and Arnold 1983). For the case of two traits (say, resistance i and competitive ability j):

$$S_i = \mathrm{cov}\left(z_i, w\right) = \mathrm{cov}\left[z_i, \left(a + \beta_i z_i + \beta_i z_j + e\right)\right] \tag{5.3}$$

Thus, for two traits, selection the differentials are:

$$S_i = cov\left(z_i, w\right) = \beta_i \cdot var\left(z_i\right) + \beta_j \cdot cov\left(z_i z_j\right) \tag{5.4}$$

and

$$S_j = cov\left(z_j, w\right) = \beta_i \cdot cov\left(z_j z_i\right) + \beta_j \cdot var\left(z_j\right). \tag{5.5}$$

The structure of variance-covariance matrix (*var-cov*) of the traits is relevant to infer evolutionary change or its constraints. For instance, evolutionary response of a trait (z_i) is not possible when no genetic variance is present in the population; on the other hand, correlated response to selection of two traits (z_i, z_j) is possible if these are genetically correlated (Falconer and Mackay 1996: see Franks et al. 2012).

Natural Selection on Defense Traits in Native and Non-native Plant Populations

Measurements of natural selection have been obtained in two plant species, an annual herb, *Datura stramonium* (Solanaceae) (Valverde et al. 2015; Castillo et al. 2019), and a perennial tree, *Melaleuca quinquenervia* (Myrtaceae) (Franks et al. 2008, 2012). *Datura stramonium*, native to Mexico and introduced to Europe after the conquest of Mexico in the fifteenth century (see Valverde et al. 2015), is found today around the world (Weaver and Warwick 1984; van Kleunen et al. 2007). In a field study, populations of *D. stramonium* were sampled in Mexico (native range) and Spain (non-native range) to measure, in each range, resistance to herbivores (relative resistance = 1 – relative damage) and plant size (basal stem diameter) (Valverde et al. 2015) and the concentration of atropine and scopolamine (Castillo et al. 2019), two tropane alkaloids implicated in plant defense to herbivores. Herbivory damage in the introduced range was caused mainly by generalist herbivores whereas in the native range by specialist herbivores. Variables (x_i) were standardized (z_i) and plant fitness (w_i) was relativized (i.e., total number of fruits per plant divided by the corresponding population mean). Standardized partial linear selection gradients (β_i; Lande and Arnold 1983) were obtained by fitting multiple linear regressions of relative plant fitness as a function of plant resistance and plant size (Valverde et al. 2015) and the concentration of atropine and scopolamine (Castillo et al. 2019) in each population.

The results indicated that plants of *D. stramonium* in the introduced range had significantly lower levels of herbivory and were significantly larger than plants in the native range (Valverde et al. 2015). In both ranges, positive phenotypic selection on plant size was detected, although higher gradients were found in the introduced range (Table 5.2; Valverde et al. 2015). Also, selection of resistance was detected in both ranges, two populations in the introduced range and four in the native range. However, a meta-analysis of selection gradients revealed a consistent and significative trend to positively select plant resistance in the native range (Valverde et al. 2015). Selection analysis of tropane alkaloids for the same populations (Castillo et al. 2019) revealed that atropine concentrations was selected against in both ranges, whereas scopolamine was selected positively (Table 5.2). A meta-analysis of selection gradients indicates that selection against atropine and positive for scopolamine were significant only in the native range (Castillo et al. 2019). These results are in agreement with the evolution of defense in the absence of natural enemies, however, given that the study was carried out in natural field conditions, environmental factors, other than herbivores, may vary greatly between ranges, potentially affecting the expression of phenotypic defensive traits (i.e., plasticity). Further studies would elucidate the role of herbivores in the evolution of plant defense in the introduced range of *D. stramonium*.

The study of *M. quinquenervia* is the first one to apply the methods of quantitative genetics and analysis of natural selection to test the predictions of EICA in an invasive plant (Franks et al. 2008, 2012). This is a common garden experiment where genotypes of the native and non-native populations of *M. quinquenervia* were grown in the introduced range (Florida USA) in the presence and absence of herbivores. *Melaleuca quinquenervia* is a tree native to Australia and invasive in Florida. It was introduced in 1800. Two insect species from Australia were introduced to serve as biological control of this tree species, a folivore (in 1997) and a sap-feeder (in 2002). The study started in 2003. Sixty maternal families of each origin (native and non-native) populations were planted in a common garden in natural conditions; a split-plot design was used to allocate half of the plants of each origin (one per maternal family) to a treatment with the exclusion of herbivores (mimicking the native habitat) and with herbivores. Each plant was recorded for resistance (presence/absence of damage) and competitive ability (rate of stem elongation) and fitness (total above ground biomass) (Franks et al. 2008). Selection gradients on resistance were all non-significantly different from zero in the presence and absence of herbivores (Table 5.2). In contrast, directional selection gradients were all positive and significant and of the same magnitude between origins and herbivore treatment (Table 5.2). No correlational or quadratic selection was detected. Results contrast with predictions based on EICA, since in the presence of herbivores there were no benefits of resistance, whereas growth rate did not increase in the absence herbivores (Franks et al. 2008).

Franks et al. (2012) sampled half of the plants of *M. quinquenervia* to analyze the concentration of 20 terpenoids in order to explore the multivariate evolution of secondary compounds, under the same experimental conditions. They found that native populations had higher concentration in 11 terpenoids than introduced plants but the latter produced significantly more E-nerolidol. Selection gradients of three compound (1,8-cineole, E-nerolidol, and viridiflorol) were estimated. No significant selection gradient was detected on viridiflorol in any combination of origin and herbivores. Positive phenotypic selection of 1,8-cineole was detected only for introduced population irrespective of presence/absence of herbivores, whereas E-nerolidol was selected positively in all conditions (Table 5.2). These, and other multivariate analysis results, suggest that the evolution of terpenoids in *M. quinquenervia* not necessarily are related to enemy release and do not offer support for EICA. Genetic correlation among traits, genetic drift and changes in the composition of herbivores between ranges may have played a role (Franks et al. 2012).

Table 5.2 Standardized genotypic (g) and phenotypic (p) directional selection gradients (β) on defense (resistance and chemical compounds) and competitive ability (growth and size) traits in native and non-native plant populations

Plant species	Range	Origin (population)	Study condition	Plant traits	β
Melaleuca quinquenervia (g)(1)	N	Australia	CG/HP	Resistance	−0.06
				Growth	**0.17**
	N	Australia	CG/HA	Resistance	−0.06
				Growth	**0.19**
	NN	U.S. (Florida)	CG/HP	Resistance	−0.03
				Growth	**0.24**
	NN	U.S. (Florida)	CG/HA	Resistance	0.03
				Growth	**0.23**
Melaleuca quinquenervia (p)(2)	N	Australia	CG/HP	1,8-Cineole	0.114
				E-Nerolidol	**0.300**
				Viridiflorol	−0.014
	N	Australia	CG/HA	1,8-Cineole	0.094
				E-Nerolidol	**0.558**
				Viridiflorol	0.015
	NN	USA (Florida)	CG/HP	1,8-Cineole	**0.424**
				E-Nerolidol	**0.293**
				Viridiflorol	−0.153
	NN	USA (Florida)	CG/HP	1,8-Cineole	**0.243**
				E-Nerolidol	**0.257**
				Viridiflorol	−0.081
Datura stramonium (p)(3)	N	Mexico (Acolman)	FS	Resistance	**0.336**
				Diameter	**0.337**
		Mexico (Patria Nueva)	FS	Resistance	**0.343**
				Diameter	0.042
		Mexico (San Martin)	FS	Resistance	0.145
				Diameter	**0.376**
		Mexico (Sanabria)	FS	Resistance	0.025
				Diameter	**0.451**
		Mexico (Santo Domingo)	FS	Resistance	**0.225**
				Diameter	**0.418**
		Mexico (Tzin Tzun Tzan)	FS	Resistance	0.018
				Diameter	**0.488**
		Mexico (Valsequillo)	FS	Resistance	**0.324**
				Diameter	0.063

(continued)

Table 5.2 (continued)

Plant species	Range	Origin (population)	Study condition	Plant traits	β
	NN	Spain (Bolonia)	FS	Resistance	0.271
				Diameter	**0.927**
		Spain (Cabeza La Vaca)	FS	Resistance	0.231
				Diameter	**0.877**
		Spain (Cardeña)	FS	Resistance	−0.157
				Diameter	**0.667**
		Spain (Castañuelos)	FS	Resistance	−0.307
				Diameter	**0.716**
		Spain (Don Fadrique)	FS	Resistance	0.175
				Diameter	**0.978**
		Spain (El Pedroso)	FS	Resistance	−0.045
				Diameter	**0.945**
		Spain (Gerena)	FS	Resistance	0.026
				Diameter	**0.551**
		Spain (Hinojos 1)	FS	Resistance	0.074
				Diameter	**0.827**
		Spain (Hinojos 2)	FS	Resistance	**0.167**
				Diameter	**0.365**
		Spain (Lora del Río)	FS	Resistance	0.036
				Diameter	**0.545**
		Spain (Pinilla)	FS	Resistance	−0.004
				Diameter	**0.810**
		Spain (Valdeflores)	FS	Resistance	0.123
				Diameter	**0.532**
		Spain (Zubia)	FS	Resistance	**0.480**
				Diameter	−0.013
Datura stramonium (p)(4)	N	Mexico (Acolman)	FS	Atropine	**−0.816**
				Scopolamine	0.576
		Mexico (Patria Nueva)	FS	Atropine	−0.064
				Scopolamine	**−0.424**
		Mexico (Santo Domingo)	FS	Atropine	**−0.576**
				Scopolamine	**0.668**
		Mexico (Tzin Tzun Tzan)	FS	Atropine	**−0.523**
				Scopolamine	**0.524**
	NN	Spain (El Pedroso)	FS	Atropine	**−0.513**
				Scopolamine	0.135
		Spain (Hinojos 1)	FS	Atropine	**−0.972**

(continued)

Table 5.2 (continued)

Plant species	Range	Origin (population)	Study condition	Plant traits	β
				Scopolamine	**0.707**
		Spain (Zubia)	FS	Atropine	**−0.564**
				Scopolamine	0.263

Modified from Franks et al. (2008) (1), Franks et al. (2012) (2), Valverde et al. (2015) (3) and Castillo et al. (2019) (4)

Significant selection gradients in bold type face. In the case of *Datura stramonium*, we show only those populations where at least one significant selection gradient was detected

N Native, *NN* Non-native, *CG/HP* Common garden/Herbivores present, *CG/HA* Common garden/Herbivores absent, *FS* Field study

However, studies in *M. quinquenervia* are highly valuable for the study of evolution of invasive plant species, revealing complex scenarios where herbivores are one of the different causes of evolution. By the analysis of the variance-covariance matrices, a better understanding of the role of drift and selection can be gained. Also, further studies addressing the analysis of the selection agents (Atwood and Meyerson 2011) and the mode in which selection affects the phenotypic and genetic variance on introduced and native ranges of invasive species are needed (Franks et al. 2012).

Concluding Remarks

The study of the evolution of plant defense under the hypotheses of enemy release has produced a wealth of research of invasive species. Different meta-analyses have found mixed support for the predicted outcomes. Importantly, derived from such analyses, this synthesis has produced recommendations for a better attack of the questions in relation to enemy release, like the nature (what kind) of genetic variation available for introductions, and the role of selection (e.g., Bossdorf et al. 2005; Colautti and Lau 2015; Dlugosch et al. 2015; van Kleunen et al. 2018; Zhang et al. 2018).

Successful invasion may depend on different factors besides herbivores and constitutes a natural experiment where evolutionary divergence has taken place very rapid (Sekor and Franks 2018b), despite we are unaware of the many failures. Data confirm that the conditions necessary for natural selection to drive the evolutionary change of invasive species are met in many instances. However, besides genetic variation, the analysis of the genetic correlations among traits is needed (Franks et al. 2012). More studies of this kind are in its beginnings. Besides its contribution to test evolutionary hypotheses, the study of invasive species has deep implications for conservations of biodiversity on Earth.

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Part II
Community Ecology of Interactions

Chapter 6
Herbivory in Cacti: Fitness Effects of Two Herbivores, One Tending Ant on *Myrtillocactus geometrizans* (Cactaceae)

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Abstract Plants in the cacti family are almost entirely restricted to the American continent; one third of the species are endemic to Mexico and many of them are threatened, with herbivory being one of the factors affecting their species populations. A range of herbivores has been reported for cacti, ranging from those that consume tissues from the stems, flowers, pollen, fruits and seeds to those that feed on fluids such as sap and nectar. Although the occurrence of this interaction is well documented, relatively few studies have assessed its effect on the plants' demography and fitness; even fewer studies have examined how the interaction of herbivores with other species (competitors, mutualistic species, predators, etc.) affects the plants' growth and net performance. This chapter briefly reviews the occurrence and effects of herbivory on cacti and how this is affected by the presence of other species and interactions. In addition, we use a biological system associated with the garambullo cactus *Myrtillocactus geometrizans*—two phytophagous species and other insects—to show how, depending on the phytophagous species and its interactions with other species also associated with the plant, the final effects on the growth, reproductive success and progeny's quality of *M. geometrizans* vary. We found that both phytophagous species affect the plant negatively, but with different intensity. The soft scale *Toumeyella martinezae* often has a greater negative effect on *M. geometrizans*, affecting its growth, reproductive effort and progeny's quality and performance. We also showed that the two scale insects compete asymmetrically: Under natural conditions, the soft scale affects *Opuntiaspis philococcus* more heavily, but the competitive advantage of *T. martinezae* is facilitated by the presence of the mutualistic ant *Liometopum apiculatum*. Thus, by eliciting a high abundance of the soft scale, the species that most affects the plant, the ant has a negative net effect on the plant. The parasitoids found in this system would compete with the ants for the phytophagous resource and, although they have a "moderate" effect

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(25% incidence) on the herbivore populations, they would favour the plant by reducing the population of phytophagous insects. The complex multitrophic system hereby studied clearly shows that analysing interspecific interactions by examining only species pairs in isolation is insufficient to understand and predict the overall impact on plants.

Keywords Competition between herbivores · Multitrophic interactions · Mutualism · Parasitoidism · Seed production · Germination · Seedling establishment · Seedling growth

Species in the Cactaceae family are distributed almost exclusively in the Americas; the family includes some 1480 species (Anderson 2001; Goettsch et al. 2015), 700 of which can be found in Mexico and almost 80% of those are endemic to the country. Species in this family display three main growth forms: cladodes, columnar and globose (Bárcenas et al. 2011). Globose and depressed globose forms are the most abundant ones; the genus *Ferocactus* is the most species-rich and the genus *Mammillaria* is the one most widely distributed (Vázquez-Sánchez et al. 2012).

The official Mexican standard NOM-059-SEMARNAT-2010—the official listing of the conservation status of Mexican plant and animal species—lists 27 cacti species as endangered of extinction, 88 threatened and 165 subject to special protection. Major pressures on these species are illegal extraction and trade of wild specimens, overexploitation of specimens or parts of them (e.g. flowers, fruits, cuttings, etc.), loss of populations caused by land use changes associated to human activities and interactions with other species, especially exotic species that negatively affect their survival and reproduction (Arias et al. 2005). Although several review studies of herbivores that feed on cacti are available (e.g. Mellink and Riojas-López 2002; Zimmermann and Granata 2002), few of those have examined the effect of herbivores on wild cacti under natural conditions, as most studies have been conducted on invasive or cultivated cacti, particularly in the genera *Opuntia* and *Hylocereus* (Bashan et al. 1995; Hoffmann et al. 1998; Palafox-Luna et al. 2018). Even fewer studies have assessed the effect of herbivory on the growth rate and fitness of cacti populations (Blom 1987; Burger and Louda 1995).

Pathogens infecting endangered or threatened cacti species (which usually have small populations)—as a side effect of consumption by herbivores—may increase the cacti's vulnerability as they reduce their population size and genetic variability, making the populations more susceptible to demographic fluctuations (De Castro and Bolker 2005). In this context, information on the demographic implications of herbivory is key for designing conservation or management plans.

Herbivores interact with plants in different ways, and their effects depend on both the plant structures they consume (e.g. vegetative or reproductive structures) and the herbivores' behaviour. A wide range of herbivores has been reported for cacti, ranging from those that consume tissues from the stems, flowers, pollen, fruits and seeds to those that feed on fluids such as sap and nectar.

Tissue Herbivores

The modification of cacti leaves into thorns and their thick cuticle are usually interpreted as adaptations to reduce water loss; however, they also represent a not entirely effective defence mechanism against herbivory as several vertebrates and invertebrates consume their tissues with various effects on the plants. In large cacti (e.g. large columnar cacti or those constituted by numerous cladodes), the consumption of some amount of tissue would have a much smaller effect than in small globose cacti, such as *Mammillaria* spp., in which herbivory has been documented to seriously affect survival and reproduction (Martínez-Ávalos et al. 2007; Hayes et al. 2013).

A wide range of herbivores feed on cacti tissues. In a review of the genus *Opuntia*, Mellink and Riojas-López (2002) reported 89 vertebrate species (mainly mammals), some 40% of which fed on trunks, stems or roots. For the globose cactus *Astrophytum asterias*, herbivory by the squirrel *Spermophilus mexicanus* has been reported to increase the mortality of adult plants between 17% and 33% (Martínez-Ávalos et al. 2007). For this same species, herbivory by beetles in the Cerambycidae group has been reported to reduce population size by nearly 50%. In this case, the damage caused was due to the tissue consumption itself and to the associated entry of pathogens into the plant.

At the Parque Provincial Ischigualasto-Talampaya in Argentina, herbivores affecting the columnar cactus *Echinopsis terscheckii* include the guanaco (*Lama guanicoe*)—the natural herbivore of this cactus species—plus feral animals such as donkeys, cows and horses that have been introduced into the area and consume intensely their tissues (Malo et al. 2010). 61.4% of the 210 *E. terscheckii* individuals examined showed some degree of herbivory by those animals, which significantly reduced their growth as well as flower and fruit production (Peco et al. 2011). A similar outcome was found in the species *Coryphantha werdermannii*: Herbivory by cows reduced its population growth rate as the animals consume the apex of adult plants, thus reducing flower and fruit production and, in general, the individuals' survival (Portilla-Alonso and Martorell 2011).

Internal feeders predominate among tissue-consuming insects; in their review of herbivorous insects of cacti, Zimmermann and Granata (2002) found that 75% of the species recorded were internal feeders, in comparison to only 3% external feeders. This feeding habit predominates particularly during the insects' larval stages and has been interpreted as an advantage of endophagy given by the protection that the plant epidermis provides against predators and parasitoids. Damage caused by mining insects can have a serious impact on populations. Oliveira et al. (1999) mention that although the damage caused by mining diptera on cladodes of *Opuntia* *stricta* remains unclear, their results show that this type of herbivory on cladodes could be negatively related to the plant's fitness.

Although much less frequent, external feeding insects can also cause significant damage, both by direct tissue consumption and by facilitating the entry of pathogens.

The reduction of the plant's fitness is what determines the nature of its response, triggering defence mechanisms that either decrease the probability of attack by herbivores (such as the production of alkaloids) or increase the plant's resistance to attacks (such as terpenes) (Marquis 1992); there are few reports of active defences (secondary metabolites) against herbivory in cacti (Farrell et al. 1991). For example, the presence of compounds such as peniocerol, macdougallin and chichipegenin in *Myrtillocactus geometrizans* might have an insecticidal effect (Céspedes et al. 2005). An extract from *Mammillaria huitzilopochtli* had antifungal effects (Robles-Zepeda et al. 2009); in addition, latex commonly present in plants of this genus can also have a defensive function against attacks (Farrell et al. 1991). Chemical and physical defences impose a cost to the plant, and they seldom occur simultaneously. Janczur et al. (2014) mention that there is a trade-off between different types of plant defences, as maintaining two defence systems consumes resources that could be allocated to growth or reproduction. There exist, however, some species that have both chemical and physical defences, but pay a very high toll. For example, when *Opuntia robusta* plants produced both chemical (phenolic compounds) and physical (formation of spines and areolas) defences, there was a high cost in terms of reproduction: 60% of hermaphrodite and 65% of unisexual plants did not reproduce in that year.

Phytophagous Insects

An effective feeding strategy of herbivores to deal with the chemical defences of some plants is by sucking fluids directly from leaves, buds, stems and roots (Celorio-Mancera and Labavitch 2016). Several species in the Hemiptera and Diptera orders, such as bugs, aphids and scales, maintain this feeding habit from juvenile forms to adults and are known generically as phytophagous insects.

Phytophagous insects often show low mobility and their immature stages determine where their development occurs; they have, therefore, limited opportunities to move on to other hosts (Wennström et al. 2010) so that their effects on host plants are highly localized. A number of evolutionary strategies that allow insects to handle the toxicity of secondary metabolites present in the plants they consume have been reported. For example, terpenoids and isoquinoline alkaloids (Berenbaum 1986; Brattsten 1986) that accumulate in the photosynthetic layer under the first protective layer of columnar cacti have toxic effects on *Drosophila* flies. However, microorganisms such as bacteria and yeasts that develop in the affected areas reduce the allelochemicals' toxicity by transforming them to non-toxic forms or by using them as energy sources, thereby reducing their concentration (Fogleman and Danielson 2001). In other cases—e.g. in various beetle species—where herbivores cannot cope with the plants' chemical defences, they inflict small wounds in the plant tissue, let the metabolites to drain out and consume the tissue afterwards (Evans et al. 2000). Phytophagous insects reported for cacti include several Hemiptera species in the Coreidae and Miridae families which are mainly associated with the genera *Opuntia* and *Nopalea* (Rocha et al. 2017). Species in the genus *Chelinidea* are the most common ones; adults and nymphs of these species are usually found feeding at the fruit's insertion point on the cladode, causing fruit loss when they form large colonies. Other bugs such as *Narnia* sp. end their life cycle on cladodes (Mann 1969; Oliveira et al. 1999; Mead and Herring 2011). Heavy infestations indirectly cause the entry of fungi on the plant's tissues, leading to their death a few months after infestation (Mead and Herring 2011).

In the group known as scale insects (superfamily Coccoidea), the best documented case is that of the genus *Dactylopius*, which has been reported for several host cacti such as *Opuntia*, *Nopalea*, *Cylindropuntia* and *Grusonia* (Britton and Rose 1963; Bravo-Hollis and Sánchez-Mejorada 1978; Brummitt and Powell 1992; Anderson 2001). This insect is locally known in Mexico as *grana cochinilla* as it produces carminic acid, the colouring principle of carmine pigment (Cortés et al. 2005). Despite its economic importance, it is also a serious pest of various *Opuntia* species (Chávez-Moreno et al. 2009). For example, it has been observed that cladodes and fruits of *Opuntia ficus-indica* drop out in the early stages of plant development, directly affecting the plant's fitness (Chávez-Moreno et al. 2009; Vanegas-Rico et al. 2010).

Scale insects in the families Pseudococcidae, Coccidae and Diaspididae (García Morales et al. 2016) have been reported to attack *Opuntia* species (locally known as *nopal*). The species *Diaspis echinocacti* is considered as an important pest of *nopal* crops (Russo and Siscaro 1994; Coronado et al. 1998; Miller and Davidson 2005; De Souza Born et al. 2009) as it reduces reproduction and survival by up to 50% (Japoshvili et al. 2010).

Folivores

Folivores are herbivores that consume buds, flowers or developing seeds (Krupnick and Weis 1999; Krupnick et al. 1999; McCall and Irwin 2006). Folivory can have direct or indirect negative effects on plants' reproductive success (McCall and Irwin 2006). Direct effects are caused by the reduction in the number of buds, flowers, fruits or seeds that plants consumed are able to produce (Oguro and Sakai 2009). Indirect effects come from changes in pollinator behaviour elicited by the reduction of floral rewards or alteration of flower structure (Krupnick et al. 1999; Cascante-Marín et al. 2009; Malo et al. 2010).

A wide variety of folivores feeding on cacti has been reported, ranging from mammals (including man) to insects. In several states of Mexico, human populations use flowers of different cacti species as food, in religious ceremonies or for their medicinal properties. For example, flowers and buds of *Lophophora williamsii* (locally known as *peyote*) are used by the Huichol people in Nayarit, Durango, Nuevo León and Zacatecas in religious ceremonies and for their medicinal properties; they are also used in meditation and psychotherapy (Narváez et al. 2018). Flowers of *Myrtillocactus geometrizans* (locally known as garambullo) as well as the fruits of *Neobuxbaumia tetetzo* and *Ferocactus pilosus* (locally known as *cabuches*) are used for culinary purposes in various parts of the country, including the states of Hidalgo, Puebla, and Durango.

Small mammals, such as the capuchin monkey, *Cebus apella*, consume buds as well as immature and mature flowers of *Pereskia aculeata* and *Rhipsalis* sp. in Brazil (Galetti and Pedroni 1994). *Mazama americana* and *Pecari tajacu* have been reported to consume flowers of *Echinopsis rhodotricha* (Nóbrega et al. 2016). The rodent *Neotoma albigula* has been reported to cause heavy damages by herbivory to *saguaro* (*Carnegiea gigantea*) plants. Out of 158 plants recorded in Arizona, 44% showed damages caused by this rodent (Hayes et al. 2013), which led to a 30% reduction in the number of flowers and 42% in the number of fruits.

Birds are also important folivores of some cacti populations. For example, 19 of the 23 species recorded in the Galapagos Islands have been reported to interact with flowers, including the finch *Geospiza fortis*, which feeds on flowers of the endemic cactus *Opuntia galapageia* reducing its reproductive success. This may be due to the scarcity of food resources (such as insects or other arthropods) in the area (Traveset et al. 2015).

Both mature and immature stages of insects are also important folivores of cacti. Lepidopteran larvae have been recognized as large consumers of reproductive structures (flowers, fruits or developing seeds) (Pratt and Pierce 2001; Ehlers and Olesen 2003; Winotai et al. 2005). Miller (2008) reported that larvae of *Cahela ponderosella* (Lepidoptera: Pyralidae) feed on the flowers and ovarian chambers of *Opuntia imbricata*, while adults of *Narnia pallidicornis* (Hemiptera: Coreidae) feed primarily on flower buds causing floral abortion. On the other hand, Martínez Peralta and Mandujano (2012) reported that beetles in the Tenebrionidae and Meloidae families feed on the flowers of *Ariocarpus* spp., while several species of orthoptera feed on the perianth and sometimes on sexual organs. Likewise, Cornejo-Romero et al. (in process) reported on beetles in the Nitidulidae family feeding on pollen, as well as several formicid species—including *Camponotus rubrithorax* and *Pogonomyrmex barbatus*—consuming perianth segments and reproductive structures (stamens and stigma) of *Mammillaria magnimamma*.

Few of the aforementioned studies evaluated the effect of folivores on cacti demography. However, Miller et al. (2009) pointed out that this is an important aspect to consider; they found that when both *Moneilema appressum* (Coleoptera: Cerambycidae) and *Gerstaekeria* sp. (Coleoptera: Curculionidae) feed on reproductive structures of *Opuntia imbricata*, its population growth rate changes considerably, although the magnitude of this effect depends on the context in which the interaction occurs, specifically on the environmental conditions, the herbivore involved, and whether this occurs alone or interacting with other organisms.

Frugivores and Seed Predators

Frugivory and seed predation are commonly seen as separate processes: the former as a mutualistic interaction where the plant invests resources in producing attractive fruits which, when consumed by an animal, this can, incidentally, transport the seeds favouring the establishment of new individuals; seed predators negatively affect plants as they reduce the number of seeds available and, thus, the possibility of new recruitments (Styrsky and Eubanks 2007; Rosumek et al. 2009). However, these two processes often occur simultaneously, and their actual net effect under natural conditions is not simple, but depends on several factors (Hulme and Benkman 2002). Seed-dispersing animals can play a very important role in the maintenance of plant populations, favouring their demography and evolution, as they bridge plant reproduction and the ensuing recruitment of new individuals.

The net effect of frugivores on plant reproduction depends on both the quantity and quality of the interaction (Schupp et al. 2017). Quantitative aspects of the interaction include, for instance, the frequency of visits or number of fruits removed; qualitative aspects include the positive/negative effects on the seeds of being consumed and dispersed by an animal.

Contreras-González and Arizmendi (2014) examined the effect that the consumption of fruits of the columnar cactus *Neobuxbaumia tetetzo* by seven bird species has on seed germination. They found that three of the species (including the two that most frequently visited and removed the most fruits) damaged the seeds so much that prevented their germination, thus making the effectiveness of mutualism nil. In other instances, the passage through the digestive tract not only does not damage the seeds but actually favours their germination. For example, Pérez-Villafaña and Valiente-Banuet (2009) reported that out of 22 bird species that visit, remove and consume fruits of *Myrtillocactus geometrizans*, the passage through the digestive tracts of ten of them increased germination rate, although only two of those species (*Phainope planitens* and *Melanerpes hypopolius*) could be considered as effective dispersers when the quality of the sites where the seeds were deposited was factored in.

Cacti dispersers include not only birds; mammals such as bats (Godínez-Álvarez et al. 2002) and even reptiles such as the lizard *Tropidurus semitaeniatus* (which consumes and disperses seeds of the shrubby cactus *Pilosocereus gounellei* in Brazilian dry forests, Gomes et al. 2016) have also been reported.

Evaluations of the dispersers' quality seldom take into account the effect of the site where the seeds are deposited (Schupp et al. 2017). Contreras-González and Arizmendi (2014) and Pérez-Villafaña and Valiente-Banuet (2009) showed that the range of potential dispersers is drastically reduced if the quality of the site where the seeds are deposited is factored in. However, García-Chávez et al. (2010) reported that seed predation can be more intense in good-quality microsites and, thus, this factor should also be taken into account when evaluating a disperser's effectiveness. Numerous studies have shown the importance of microhabitat and the presence of nurse species for the successful establishment of cacti seedlings (Valiente-Banuet and Ezcurra 1991; Flores et al. 2004;); this component is key for elucidating its real effect on the plant population.

Although vertebrates are regarded as the main frugivores of cacti (Contreras-Godínez-Álvarez et al. 2002; González and Arizmendi 2014; Vázquez-Castillo et al. 2019), other animals such as ants often visit the fruits of cacti (Luna et al. 2018) and can act as their primary dispersers (Munguía-Rosas et al. 2009).

Few studies on frugivory and seed predators have evaluated the demographic consequences for the plant. Godínez-Álvarez et al. (2002) examined the effect of seven species of seed dispersers on the population growth rate of the columnar cactus *Neobuxbaumia tetetzo* using matrix models. They found that the bat *Leptonycteris curasoae* had the greatest positive impact on the population growth rate, considering the number of fruits removed, the effect on the germination of seeds that had passed through their digestive tract and the places where the seeds were deposited. This bat was also the only disperser with which the cactus population growth rate (λ) was greater than 1.

Although corroborating information is necessary, evidence available indicates that frugivores and seed predators can influence not only plant demography but also the evolution and selection of life history characteristics (Hulme and Benkman 2002), mainly because plants invest resources in producing fruits that are attractive for animals to consume and, thus, disperse their seed (Holland and Fleming 1999).

The interaction between a seed predator and a plant can have different outcomes depending on the environmental conditions where it occurs, as these can modify the interaction intensity by, for example, changing the number of seeds predated or the probability of seedling establishment (Vila and Gimeno 2003; Ford et al. 2015; Miranda-Jácome and Flores 2018).

Tritrophic Interactions and Their Implications for Herbivory

Most studies on herbivory have examined the plant and its herbivore in isolation (Halpern and Underwood 2006; Myers and Sarfraz 2017). However, current efforts aim to understand how other interactions that herbivores establish with other organisms in the system can ameliorate or intensify herbivory and, thus, modify the direct or indirect, positive or negative effects on the plant. Although it is difficult to find a system in which the effects of phytophagous insects on the plant's demography can be evaluated, at the same time identifying the factors that modulate such effects, some cases have been reported that show the importance of widening the studies' scope to also include other trophic levels (Agrawal 2004; Maron and Crone 2006; Miller 2008).

Natural Enemies (Predators, Parasites and Parasitoids) as Mediators of Herbivory

The density of herbivore populations tends to decrease when controlled by their natural enemies (predators or parasitoids) (Gibernau and Dejean 2000; Ozaki et al. 2000; Schmitz et al. 2000). Thus, their negative effects on the plants' reproductive success are reduced (Abdala-Roberts et al. 2012).

Predators can affect plant performance by influencing herbivore abundance. Stefani et al. (2015) and Koptur et al. (2015) found that predatory spiders favoured the host plant's reproductive success. Such positive effect was more intense when ants that visited and fed on extrafloral nectaries were absent, as they also drove herbivores away. The presence of predators does not necessarily translate into benefits for the plant. Miller (2008) reported that the abundance of the cactus bug, *Narnia pallidicornis*, which feeds on the *cholla* cactus is not affected significantly by predatory spiders. Thus, the presence of this predator does not benefit the plant.

Parasitoids are insects that, during their larval stages, feed and develop inside or on another invertebrate host, which they eventually kill (Godfray 1994; Portillo and Vigueras 1998). This interaction has been poorly documented in cacti; the few studies available have focused on the use of parasitoids for pest control in *Opuntia* spp. and *Cylindropuntia* spp. plantations. *Cactoblastis cactorum* (Pyralidae) causes serious damage to *nopal* plantations, a commercially important crop in Mexico. Parasitoids such as *Apanteles opuntiarum*, *Phyticiplex doddi* and *Brachymeria cactoblastidis*, among others, have been successfully used to control *C. cactorum* in *Opuntia ficus-indica* plantations (Goñalons et al. 2014; Palomares 2018). Another example is that of the cactus mealybug, *Hypogeococcus pungens* (Pseudococcidae), which interferes with the growth, flowering and fruiting processes in *Opuntia* plantations (Zimmermann and Pérez-Sandi 2010). This pest has been successfully controlled with parasitoids in the genus *Leptomastidea* sp. (Encyrtidae) (Ramos and Serna 2004).

Hymenopterous parasitoids are consumers in the third or fourth trophic level and play an important role in multitrophic interactions and, thus, in natural communities (Bonet 2009). Although they often go unnoticed because of their small size, they play an important role as regulators of a large number of herbivores and are, therefore, useful for managing insect pests (Vanegas-Rico et al. 2010).

Mutualism as a Mediator or Intensifier of Herbivory

Herbivores respond in different ways to selection pressures to reduce predation or parasitoidism rates. One of such responses consists of establishing mutualistic relationships with other species that provide protection against natural enemies (Völkl et al. 1999; Keller et al. 2018). Mutualistic associations between plants and animals, particularly insects, that act as pollinators or seed dispersers have been widely documented. Mutualistic associations involving plant protection in exchange for some kind of reward (either accommodation as in the case of ants inhabiting the domatia in *Acacia* plants, or food mainly provided through extrafloral nectaries) have only been recently examined (Rico-Gray and Oliveira 2007).

The association between plants and ants has been studied in a number of plant groups, commonly finding that the host plant derives some benefit either in terms of growth or reproduction (Trager et al. 2010). Rosumek et al. (2009) reviewed 81 previous studies assessing the role of ants as defence mechanism of plants. They found that the absence of ants causes significant reductions in the plants' fitness: Plants without ants suffered 97% more herbivory and average reductions of 27% in biomass, 52% in leaf production and up to 59% in flower, fruit and seed production. This effect was more intense in tropical than in temperate climates.

The association between cacti and ants has also been studied. The main mechanism underlying this association is the reward that the plant provides through its extrafloral nectaries (EFN). Miller (2007) reported that ants visiting *cholla* plants (*Opuntia imbricata*) to obtain food from EFN have a positive effect on the plant as they defend it from other herbivores and seed predators. Oliveira et al. (1999) obtained similar results with *Opuntia stricta* in coastal environments of eastern Mexico. However, it is not entirely clear whether the ants that visit and consume EFN resources always benefit the plant, even if they drive other herbivores away.

Miller (2007) found that of the two ant species that visit *cholla* plants, only one of them (*Liometopum apiculatum*) actually benefits the plant by defending it from other herbivores and seed predators. In the globose cactus *Ferocactus wislizenii*, individuals associated with ants were found to produce more fruits, and that the ants attacked herbivores that they came across on the plants. However, there were no significant differences in the population growth rate when compared to plants without ants (Ford et al. 2015).

Interactions between ants and plants can be mediated by other species, particularly herbivorous insects that feed on sap and also supply rewards for ants. In this interaction, ants are attracted by the secretion of honeydew-producing hemiptera; being this a predictable, high-quality resource, the ants tend and protect the hemiptera from predators and parasitoids (Buckley 1987).

Although the plant would endure some negative effects caused by the ant-tended herbivores, the overall net effect on the plant can still be positive. The increased predation by ants could eliminate or reduce other more harmful herbivore populations (such as other sucking or defoliating insects). By contrast, as the ants protect the hemiptera from other predators and parasitoids, their populations might grow so large that the negative costs for the plant increase.

In their review of the interaction between ants and honeydew-producing insects, Styrsky and Eubanks (2007) found that in most cases (73%) the interaction had a positive effect on the plant. However, in almost one fourth of the studies reviewed, plants were negatively affected by the presence of ants and honeydew-producing insects. Possible explanations for this include:

The damage that ants cause to reproductive structures the ants patrolling upon flowers can drive pollinators away and thus affect fruit and seed production, the population of ant-tended honeydew-producing insects might become so large that affects the plants negatively, the possibility of these insects to transmit diseases.

The final net effect of the plant-ant-hemiptera interaction on the plant will depend on the balance between these positive and negative effects (Rosumek et al. 2009; Vilela and Del-Claro 2018). Styrsky and Eubanks (2007) also pointed out that the interaction between ants and honeydew-producing insects (and, consequently, with the plant) is far from being obligate and would be conditioned by several factors, including the presence and quality of "distractors" in the plant itself (e.g. extrafloral nectaries) which divert the ants' attention from the honeydew-producing insects (Pacelhe et al. 2019), the cost that honeydew-producing hemiptera have for the plant and the effectiveness of ants in preventing damage to honeydew-producing insects by other predators, parasitoids or competitors.

For these reasons, Rosumek et al. (2009) claim that this interaction is usually facultative, opportunistic and variable, with a high level of uncertainty as its final net effects and its constancy over time.

Competition as a Mediator or Intensifier of Herbivory

Several studies have studied competition between herbivores (Kaplan and Denno 2007), but only few of them have evaluated how this interaction affects plant performance. In a study on the *cholla* cactus, *Opuntia imbricata*, the presence of the herbivores *Narnia pallidicornis* (Hemiptera: Coreidae) and *Moneilema appressum* (Coleoptera: Cerambycidae) was negatively correlated and the plant quality affected the density of one of the competitors (*N. pallidicornis*) and, therefore, the intensity of competition. The effect of changes in the intensity of competition between the herbivores on the fitness of *O. imbricata* was not evaluated, however (Miller 2008). It is currently known that, in general, competition between herbivorous insects coexisting on the same plant is asymmetric (Kaplan and Denno 2007; Moncada 2019) and affects the insects' growth rate (Zeilinger et al. 2011).

Evidently, the end effect on the plant's growth, reproductive success and demography will depend on the type of interactions that the herbivores establish with other species occurring on the same plant (Miller et al. 2009; Quintana-Ascencio et al. 2018). Hence the importance of studying interspecific interactions encompassing at least three of the species involved (Denno et al. 1995; Miller 2008; Abdala-Roberts et al. 2012).

Case Study: *Myrtillocactus geometrizans*, Two Are Not Enough

Studying multitrophic interactions allows a better understanding of coevolutive interactions between plants and their herbivorous insects (van Dam and Heil 2011) and helps to better explain the ecological and evolutionary responses of the interacting species, which could hardly be inferred by analysing two-species interactions (e.g. the plant and the herbivore) in isolation (Heil 2008; Miller et al. 2009, van Dam 2009; Ali et al. 2012). Studying multitrophic systems also helps to identify which factors regulate (either in a bottom-up or a top-down manner) the populations and communities of herbivores on plants (Hairston et al. 1960; Oksanen et al. 1981; Hunter and Price 1992; Miller 2008) and how other interactions can mediate or intensify this interaction, herbivory, in our study case (Denno et al. 1995; Miller 2008).

The relative importance of host plants, predators and competitors on the population dynamics of herbivorous insects has been intensively studied and debated (Hunter and Price 1992). However, the joint effects of bottom-up, top-down and lateral (i.e. within the same trophic level) interactions have rarely been studied in a single system (Miller 2008).

Cacti can be a precious resource for animals in xerophytic shrublands of arid and semi-arid zones, due to the large amount of water and nutrients that they store and are almost continuously available. Among the animal groups that exploit these resources are phytophagous insects which, in turn, interact with other species including natural enemies (predators and parasitoids), mutualistic species and competitors (Callejas-Chavero et al. 2013). For this reason, plants inhabiting xerophytic shrublands, along with the species interacting with them, provide an ideal system for examining multitrophic relationships (Pérez-Villafaña and Valiente-Banuet 2009).

We have been conducting a multi-year study on the interactions between herbivory, mutualism, competition and parasitoidism relationships between different insect species associated with *Myrtillocactus geometrizans*, a cactus common in arid zones of central Mexico. In this section, we present some of the results obtained in this study to demonstrate the importance of adopting a multitrophic approach when studying ecological interactions under field conditions.

Study Species

Myrtillocactus geometrizans (locally known as garambullo) is a cactus endemic to Mexico, where it is widely distributed in xerophytic habitats (Arias et al. 1997; Hernández-López et al. 2008). It has ascending, light green or blue-green stems and can reach up to 5 m in height. Flowering occurs from February to April; flowers are greenish white. The fruits are small, globose and dark purple in colour and the fruiting season lasts approximately 3 months. Seeds are globose with a matte black testa and do not require prior treatment to germinate (Bravo-Hollis and Sánchez-Mejorada 1978; Arias et al. 1997; Hernández-López et al. 2008). Numerous interspecific interactions involving this cactus have been reported: phorophyte for epiphytes in the genus *Tillandsia* (Martínez-Hernández 2017); its seeds are dispersed by birds and mammals; it is pollinated mainly by bees (Pérez-Villafaña and Valiente-Banuet 2009; Maqueda in process); it is used as shelter and food by various arthropods which, in turn, interact with each other and with the host plant in different ways (Callejas-Chavero et al. 2013; Martínez-Hernández 2015).

Two phytophagous species feeding on *M. geometrizans* have been reported. The soft scale *Toumeyella martinezae* (Hemiptera: Coccidae) is an insect with a convex, oval body that becomes sessile after the first development stage and completes its life cycle in place. Females produce a sugary substance (honeydew) that allows them to establish a mutualistic relationship with the ant *Liometopum apiculatum* (Hymenoptera: Formicidae). The ants feed on the honeydew produced by the soft scale and, as has been reported for other similar systems, would likely protect them from natural enemies (predators and parasitoids), groom them and relocate them to places suitable for settlement (Martínez-Hernández 2015).

As of today, this interaction has only been observed in a population of *M. geometrizans* located near the municipality of Huichapan in the state of Hidalgo in central Mexico. The armored scale *Opuntiaspis philococcus* (Hemiptera: Diaspididae) has a white waxy cover; like *T. martinezae*, it becomes sessile after the first development stage and completes the rest of its life cycle on the garambullo plant. *O. philococcus* does not produce honeydew. This species has been recorded on different host species in the genera *Opuntia* and *Lemaireocereus* (Hamon 1980) and, recently, also on *M. geometrizans* (Martínez-Hernández 2015). Both scale species can be found coexisting on garambullo plants and are, in turn, used as hosts by various parasitoids: *Mexidalgus toumeyellus* and *Coccophagus ruizi* (aphelinid) are parasitoids of *Toumeyella martinezae*, whereas *Plagiomerus diaspidis* (encyrtid) is parasitoid of *Opuntiaspis philococcus* (Martínez-Hernández 2015; Islas 2020 in process) (Fig. 6.1).

In this multi-species system, we have recorded the effect of the herbivore species—either on its own or in coexistence—on plant performance variables (e.g. branch growth), reproductive effort (size of flowers and fruits, number of seeds produced) and progeny quality (seed size, germination percentage and seedling size). We have also explored the potential competition between the phytophagous species and how this can be affected by the ants' presence. Finally, we have recorded the occurrence and intensity of parasitoidism on the phytophagous species (Box 6.1).

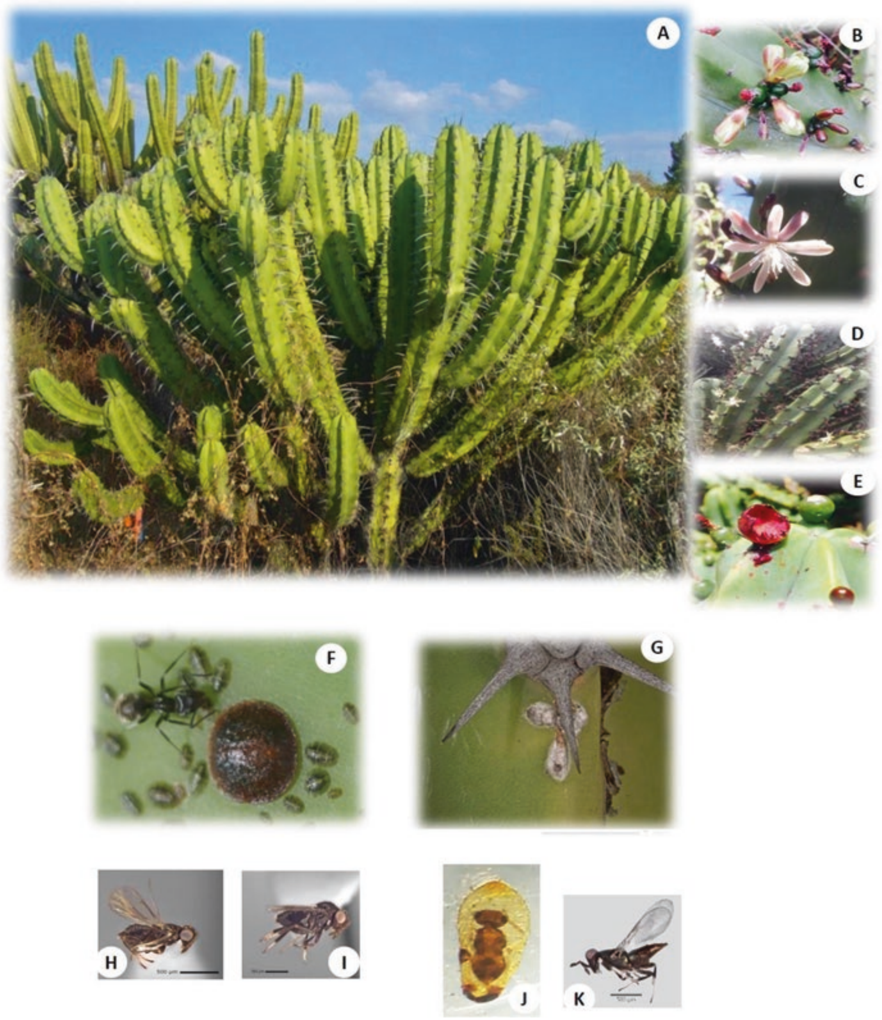

Fig. 6.1 Study system. A Myrtillocactus geometrizans (garambullo). B Flower buds emerging from areoles along the branches. C Garambullo flower. D Branch with immature fruits. E Ripe, partially eaten fruit. F *Toumeyella martinezae* (soft scale) with *Liometopum apiculatum*. G *Opuntiaspis philococcus* (armored scale). H *Mexidalgus toumeyellus*. I *Coccophagus ruizi* (afelinids) parasitoids of the soft scale. J *O. philococcus* pupa, with a parasitoid developing inside it. K *Plagiomerus diaspidis* (encyrtid), parasitoid of the armored scale

The Effect of Herbivores on Myrtillocactus geometrizans

As expected, in general we found that both herbivore species affect the host plant negatively, although the magnitude of this effect varies between species. The armored scale (*Opuntiaspis philococcus*) generally causes less damage to

Box 6.1

The study area is covered by a xerophytic shrubland containing some 0.01 *Myrtillocactus geometrizans* plants per m^2. Adult plants were identified and classified into the following classes/conditions: (i) control plants, with no scale insects; (ii) plants bearing only *Opuntiaspis philococcus* (armored scale); (iii) plants bearing only *Toumeyella martinezae* (soft scale) and its mutualistic ant, *Liometopum apiculatum*; and (iv) plants bearing both species of phytophagous scales plus the mutualistic ant.

A sample of branches was randomly selected from each plant in order to record variables describing the plant's growth and reproduction. Plant growth was estimated in terms of the increase in branch length; reproduction was estimated in terms of the size of flowers and fruits produced. A random sample of ripe fruits was taken from plants in each condition to measure their size, count the number of seeds per fruit and measure seed size (in a sample of 300 seeds per condition). A sample of 1000 seeds per condition was used to determine germination percentage; the size of 4-month-old seedlings was also recorded (Box Fig. 6.1I). Percentage of parasitoidism in each of the phytophagous species was determined (as percentage of individuals with parasitoids) by carefully examining individuals in different stages of development to identify and count the incidence of parasitoids (Box Fig. 6.1II).

To assess competition between the two phytophagous species (as well as the effect of the mutualistic ant on this interaction), 26 *M. geometrizans* individuals were selected and classified into 5 conditions: (i) plants bearing *O. philococcus* without competition; (ii) plants bearing *T. martinezae* and its mutualistic ant *L. apiculatum*, without competition; (iii) plants with *T. martinezae* without competition, but ants excluded by isolating the branches by means of a ring of solid, unscented petroleum jelly; (iv) plants bearing both phytophagous species plus the mutualistic ant; and (v) plants in which the two scale insects compete for space but with the mutualistic ant excluded (by isolating each branch with a ring of solid, unscented petroleum jelly, Box Fig. 6.1III). Plants in each condition were monitored for 7 months; the abundance of scale insects was censused every 6 weeks (Box Fig. 6.1IV).

garambullo plants than the soft scale (*Toumeyella martinezae*). Although the armored scale had no negative effect on the plant's growth (in fact, it had a slightly positive effect: 4.1%), it did affect its reproductive effort as flowers were approximately 42% smaller, on average, than flowers of control plants (without herbivores). There was also a significant reduction (17%) in the average number of seeds per fruit, the size of the seeds produced (3.4%) and their germination capacity (0.2%) and 4-month-old seedlings were 27% smaller than seedlings produced by control plants (Fig. 6.2).

Box Fig. 6.1 **I**.—*Myrtillocactus geometrizans* seedlings derived from plants with different herbivory conditions in the field. Green dots: *Toumeyella martinezae* with its mutualistic ant and *Opuntiaspis philococcus*. Red dots: *Toumeyella martinezae* with its mutualistic ant. Blue dots: *Opuntiaspis philococcus*. White dots: Control plants (without phytophagous insects). **II**.—Branch of Myrtillocactus geometrizans (garambullo) with scale insects (*Toumeyella martinezae*) killed by parasitoids. **III**.—Branches of *Myrtillocactus geometrizans* (garambullo) showing the petroleum jelly ring used to exclude ants. **IV**.—Sampling unit (subdivided wooden quadrat) used to estimate the density of *Opuntiaspis philococcus*

By contrast, the soft scale (*Toumeyella martinezae*) affected the garambullo plants more intensely in all the variables measured: Branch growth was reduced in 61% on average; flower size, fruit size and number of seeds were reduced in 49%, 19% and 17%, respectively. Also, the progeny's quality was seriously affected as seeds and seedlings were 9% and 44% smaller than those produced by control plants. The percentage of seed germination was the only variable that did not show

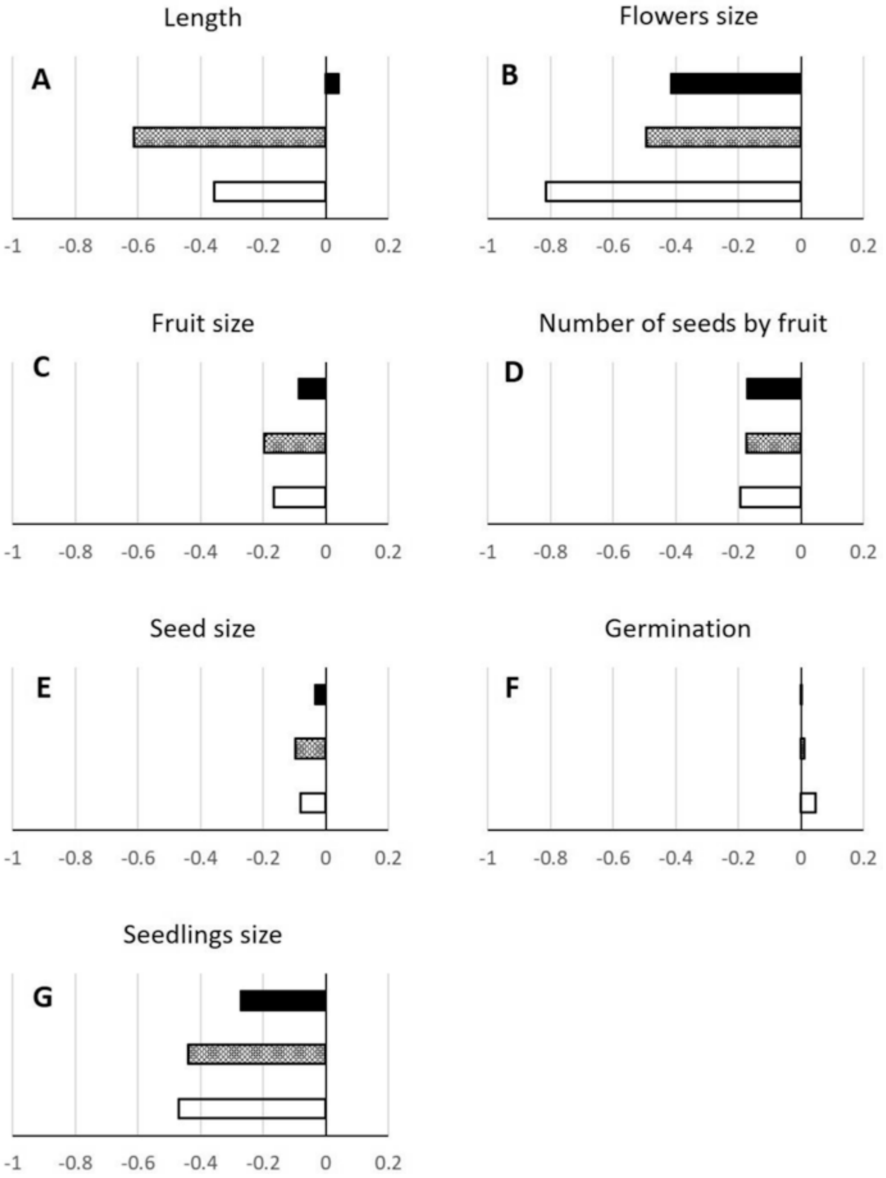

Fig. 6.2 Effect of phytophagous insects on the growth, reproductive success and progeny's quality of *Myrtillocactus geometrizans*. The x-axis shows the relative change in each variable with respect to the control condition (plants without phytophagous insects): Positive values denote effects larger than in control plants; negative values denote effects smaller than in control plants. Black bars correspond to the armored scale, grey bars to the soft scale and white bars to the condition with both scale species present

a negative effect; in fact, seed germination was slightly favoured as 10% more seeds germinated than those from control plants (Fig. 6.2).

The Effect of Competition on Herbivory

Under natural conditions, phytophagous insects do not interact only with the plant they feed upon, but they also interact both with each other and with other animal species. Depending on their nature and intensity, such interactions might affect the phytophagous insects' performance and, thus, their effect on the host plant. In our study system, both phytophagous species commonly coexist on the same plant so that some level of competition for resources is to be expected. In this case, such competition would be also mediated by the presence of a mutualistic ant as all the soft scale populations that we have examined are tended and exploited by ants. To test this, we assessed the competitive relationship between the two phytophagous species by recording the abundance of each of them on plants where they occurred either on their own or in the presence of the possible competitor. We also evaluated the effect that excluding the mutualistic ant (which, under natural conditions, is always associated with the soft scale, Box 6.1) had on this presumably competitive interaction.

Our results show that the two scale species do compete for space for settlement on garambullo plants and that the armored scale is more heavily affected by the presence of its competitor than the soft scale. The average abundance of *Opuntiaspis philococcus* decreased in 77% when coexisting with the soft scale, while the abundance of the soft scale *Toumeyella martinezae* only decreased in 28% (Fig. 6.3). The competitive advantage of the soft scales depends, to a large extent, on the presence of the ant. When ants were excluded, the abundance of the soft scale decreased in 80%, while the abundance of the armored scale increased in 13%. This shows the strong dependence that *T. martinezae* has on its mutualistic ant and how the ant's presence is vital to gain a competitive advantage on the other phytophagous species.

In our study system, competition between the two phytophagous species leads to a decrease in the abundance of both species, which could reduce the final negative effect on the plant. We have not yet examined how changes in herbivore abundance affect the plant's demography or adaptation. However, we did assess the plant's performance when both herbivores were present. When the two phytophagous species co-occurred on the garambullo plant, the latter experienced clear negative effects compared to the performance of control plants (without herbivores). As for the magnitude of such effects, the results obtained were more alike those obtained in the presence of only the soft scale than of the armored scale. In all cases when both scale species were present, the negative effects on the plant increased strongly in comparison to those observed with the armored scale only. This suggests that the presence of the additional phytophagus species has a cumulative effect. By contrast, only three of the variables examined (flower size, seed number and seedling size) showed an effect greater than that observed when only the soft scale was present, and none of the variables examined showed an effect equal or greater than the sum of the effects observed with each of the phytophagous species acting individually. These results demonstrate the existence of an interaction between the

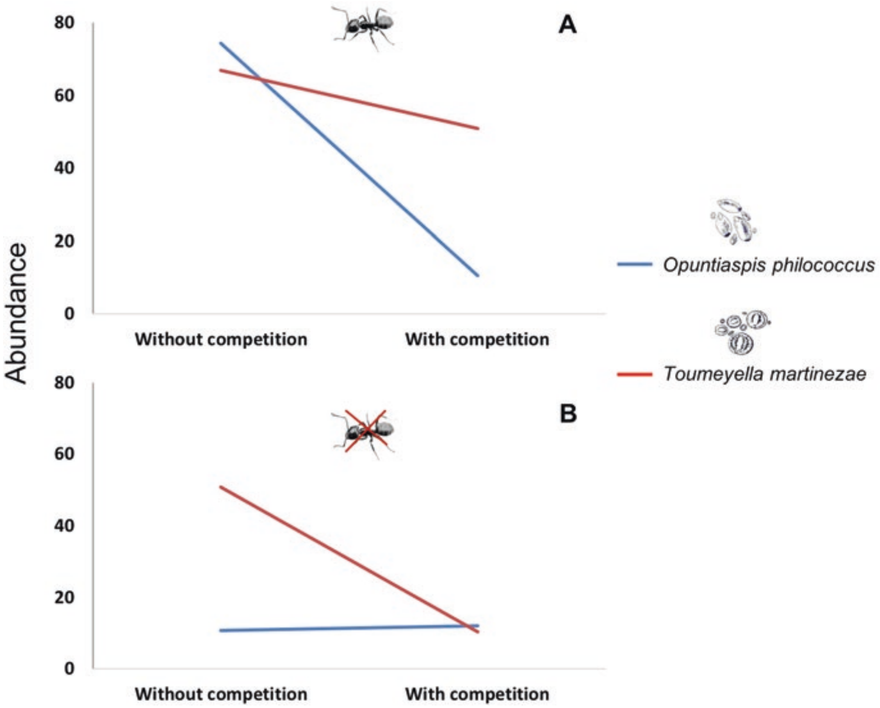

Fig. 6.3 Competition between *Toumeyella martinezae* (soft scale, red lines) and *Opuntiaspis philococcus* (armored scale, blue lines), in the presence (**a**) or absence (**b**) of the mutualistic ant *Liometopum apiculatum*

two phytophagous species which determines the final net effect on the plant: The growth of, and the size of fruits and seedlings produced by, plants in which both phytophagous species co-occurred showed a smaller negative effect than those of plants in which only the soft scale was present (Fig. 6.2). From the plant's point of view, it is better to bear the armored scale competing with the soft scale.

Effect of the Mutualistic Species on Competition and Herbivory

Several studies have documented the positive or negative, direct or indirect effects that ants can have on the plants they visit (Styrsky and Eubanks 2007; Rosumek et al. 2009). Although we have not yet experimentally evaluated this effect in our study system, some data that we have collected suggest that the presence of mutualistic ants has both positive and negative effects. Ants would affect the plant negatively because they help maintain a relatively high abundance of *T. martinezae* (the species most harmful to the plant). We have observed, however, that when populations of this herbivore become very large, the ambrosia secreted but not used by the ants facilitates the invasion of a fungus that causes branch death and, in some cases, even death of the entire plant. Therefore, regulation of herbivore populations by the ants is not entirely efficient and, from that perspective, the presence of ants does not have a positive effect on plants. In order to better understand these interactions, the effect—if any—that ants have on other herbivores and on fruit and seed production needs to be evaluated as ants are frequently observed patrolling the flowers.

The third component of this multi-species system are the phytophagous' parasitoids. The percentage parasitoidism of *Toumeyella martinezae* when not protected by

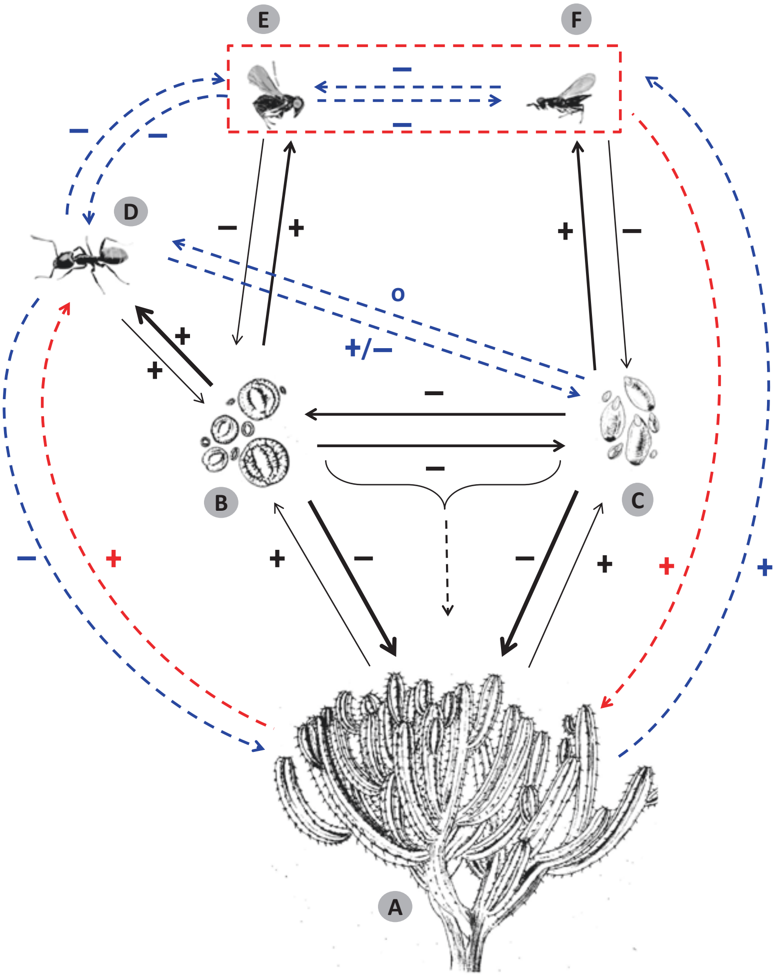

Fig. 6.4 Interspecific interactions involving *Myrtillocactus geometrizans*. Solid lines represent direct effects between the species involved; arrows denote the direction and signs the nature of each interaction (+ positive, – negative and 0 neutral). Line thickness denotes the interaction intensity. Dashed lines represent positive and negative, indirect effects that have been observed in the system, but have not been yet studied systematically. Green, dashed lines represent interactions for which our results provide evidence; blue lines represent effects that can be inferred but now are still not known

ants was estimated in 32%, but only 17% in the presence of ants. Parasitoidism in *Opuntiaspis philococcus* varies between 6% and 88%, which is positive for the plant since this reduces the phytophagous population and, therefore, herbivory pressure.

In this multitrophic system, parasitoids and ants affect each other negatively: Ants prevent parasitoids from accessing their hosts (scale insects), while parasitoids affect the survival of scale insects, thus decreasing the availability of food for ants (Fig. 6.4).

Although most of the results presented here mostly concern interactions between organisms associated with *M. geometrizans*, we have evidence that these interactions, directly or indirectly, have positive and negative effects on the plant's growth, reproductive effort and progeny's quality. We therefore conclude that *Toumeyella martinezae* is the phytophagus species more harmful to the plant, due to its mutualistic interaction with *Liometopum apiculatum*.

The complex multitrophic system studied here clearly shows that analysing interspecific interactions by examining only species pairs in isolation is insufficient to understand and predict the overall impact on plants.

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Chapter 7
The Extended Microbiota: How Microbes Shape Plant-Insect Interactions

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Abstract Microorganisms were the first forms of life on Earth and are now part of all living organisms, but the role they played during the evolution of multicellular species is still a mystery. Among other biotic interactions, plants and their herbivorous insects have always occurred under a microbial milieu. During the past 20 years, our understanding of how microorganisms shape the ecology and evolution of plant-insect interactions has increased rapidly. However, the extent to which plant-associated microbes influence insect performance and how insect-associated microbes influence plant defenses remains largely unexplored. Here, we will highlight the potential reciprocal feedbacks between the microbiotas of plants and insects that could affect their interaction. We also bring attention to how network theory can help us understand the potential interactions within and between microbiotas. Finally, we will point out some promising directions for future experimental studies in order to better understand microbe-insect-plant interactions.

Keywords Defense · Microbiota · Network theory · Plant-insect interactions · Resistance · Tolerance

Despite the historical pairwise perception of the coevolutionary process between plants and herbivorous insects, the environment surrounding plants and their consumers is far from sterile; thus all their interactions take place under a microbial milieu that can significantly alter the ecology and evolution of both plants and insects (Felton and Tumlinson 2008). Metagenomic studies have accelerated our understanding of the fundamental role played by microorganisms in the survival

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and adaptation of plants (Partida-Martínez and Heil 2011; Pineda et al. 2013) and their herbivorous insects (Chu et al. 2013; Chung et al. 2013; Asplen et al. 2014; Sharpton 2018). Nowadays, we also acknowledge that microorganisms can mediate biochemical communication between plants and insects (Hansen and Moran 2013). Indeed, the field of microbe-insect-plant interactions has been rapidly expanding over the past two decades with excellent reviews about the ecological implications of microorganisms (Biere and Bennet 2013; Biere and Tack 2013; Casteel and Hansen 2014; Sugio et al. 2015; Mason et al. 2019). However, the extent to which plant-associated microbes influence insect performance and how insect-associated microbes influence plant defenses remains largely unknown (but see Schausberger 2018 for induced resistance). Our aim is to provide a conceptual framework to fill this gap through the understanding of possible reciprocal feedbacks between the microbiotas of plants and insects that could affect their evolution. First, we will discuss how the holobiont concept came to be and whether this view actually helps us in understanding the ecology and evolution of hosts and their interactions. Next, we will review evidence about how the phyllosphere affects insect performance and possible feedbacks between the insect microbiota and plant defenses. We will finally point out how network theory can shed light into evaluating coevolutionary processes between the microbiotas of plants and insects.

From Microbes Through Holobionts to Plant-Insect Interactions

Our understanding of how microorganisms can shape the ecology and evolution of plant-insect interactions has been increasing rapidly since the last 20 years. However, along with all these advances came a general confusion in the terms and concepts frequently used in the context of the host-microbe interactions. The term holobiont was coined by Lynn Margulis (1990) to describe the intimacy between a host and its microbial symbiont. While this term was initially developed to explain the origin of eukaryotic cells, it was latter extended to include other obligatory symbioses (O’Malley 2017). At the beginning of this century, and under the umbrella of the hologenome theory of evolution, the term holobiont was redefined as a host (plant or animal) together with all its associated microorganisms upon which natural selection can operate (Zilber-Rosenberg and Rosenberg 2008; Theis et al. 2016; Rosenberg and Zilber-Rosenberg 2018). Along with this new view of holobionts, other concepts were commonly used although not always with the same meaning. Thus, in 2015 Marchesi and Ravel proposed clear definitions which we will follow throughout this review. The microbiota refers to the assemblage of microorganisms present in a defined host. The microbiome includes the host, all its microorganisms, their genomes, and the surrounding environmental conditions. The hologenome can then be defined as the sum of the genetic information of the host and its microbiota. While there is now plenty of evidence supporting the hologenome hypothesis of evolution, whether the holobiont can function as an evolutionary unit is still under debate (Moran and Sloan 2015; Douglas and Werren 2016; Doolittle and Booth 2017; Doolittle and Inkpen 2018). Holobionts are indeed evolving units, but not evolutionary units upon which natural selection can act because a holobiont is better viewed as an ecological community with interactions that range from parasitic to mutualistic, with horizontal and vertical transmission and multiple levels of fidelity among the partners.

In considering the ecology and evolution of holobionts, the fidelity in the transmission of the microbiota along generations is of the most interest. It has been then proposed that the holobiont is constituted by resident microbiota (of vertical transmission), semi-resident microbiota (of vertical and horizontal transmission), and transient microbiota (of horizontal transmission) (Roughgarden et al. 2018). While all three types of microbiota can affect its host fitness (Zakharov 2015; Hurst 2017), the resident microbiota might be more important in evolutionary terms, while the transient microbiota represents an important source of variation affecting the host ecological interactions (Callens et al. 2018; Guégan et al. 2018). In this sense, it is interesting to note that a group of microbes appear to be shared and maintained among most individuals of a single population despite spatial and temporal variation (Roeselers et al. 2011; Lowe et al. 2012; Dougal et al. 2013; Astudillo-García et al. 2017; Kwong et al. 2017). This group of microbes has been termed the core microbiota (Shapira 2016), and while there is still no clear consensus on how to delimit or measure it (Shade and Handelsman 2012; Hurst 2017), considering its function rather than its composition could prove more insightful.

The core microbiota becomes more relevant if functional groups are considered, instead of taxonomic groups, because the latter gives no information about their contribution to the host phenotype (Doolittle and Booth 2017; Foster et al. 2017; Lemanceau et al. 2017). Moreover, considering the functional core microbiota implies that those transient or horizontally transmitted microbes could eventually replace those from the core without altering the host ecology and evolution. Thus, the presence of certain specific lineages of microbes would be sufficient to allow the functional assembly of the holobiont (Roughgarden et al. 2018). This functional contribution could then be relevant even when the microorganisms do not have a common evolutionary history with their host (Catania et al. 2017). Ultimately, the (extended) phenotype expressed by a particular host is the result of not only the presence of different microbes but also their functional contribution. Because this extended phenotype is the one that interacts with the consumers, the functional core microbiota will play a role in plant-insect evolution. Thus, in the context of plant-insect interactions, it is important to understand the effects of the microbiota on its host but also on how this host interacts with other organisms. If the presence/absence of specific lineages in the microbiota affects the fitness of either the plant, the herbivore, or both, a third-party player should be recognized in the battle between plants and herbivorous insects.

The Phyllosphere and Insect Performance

The surface of the leaves is the habitat for large and diverse microbial communities defined as phyllosphere (Ruinen 1956; Lindow and Brandl 2003; Vorholt 2012). All of these microbes are, at some point, inevitably consumed by the insects. While the impact of consuming entomopathogens has been the aim of several studies (Cory and Hoover 2006; Shikano 2017), little is still known about the possible effects of consuming nonpathogenic microbes. Recent evidence suggests that phyllosphere bacteria can indeed colonize the insect midgut (Mason and Raffa 2014; Bansal et al. 2011). Actually, it has been shown that the symbionts in the midgut of the gypsy moth *Lymantria dispar* are mostly obtained from its host plant (Broderick et al. 2004). However, few studies have evaluated the effect of the phyllosphere on insect performance. To our knowledge only two studies have specifically evaluated the effect of the phyllosphere on insect performance.

Shikano et al. (2015) evaluated the effect of two common bacterial colonizers of the phyllosphere (*Pseudomonas fluorescens* and *P. syringae*) on the performance, immunity, and resistance of the cabbage looper *Trichoplusia ni*. They found that consumption of the phyllosphere bacteria decreased larval growth rate but had no effect on immunity and, while the larval resistance to a baculovirus was not affected, resistance to pathogenic bacteria was concentration-dependent. The phyllosphere, however, can also have positive effects on larval performance. Larvae of the gypsy moth were bigger when consuming diet enriched with bacteria from the phyllosphere of the quaking aspen *Populus tremuloides* compared than when consuming diet enriched with bacteria previously isolated from their own guts (Mason et al. 2014). That is, bacteria that most benefitted larvae were initially foliar residents, suggesting that toxin-degrading abilities of phyllosphere inhabitants indirectly benefit herbivores upon ingestion (Mason et al. 2014). Interestingly, herbivory can in turn influence the phyllosphere. In the plant *Cardamine cordifolia*, the abundance of *Pseudomonas syringae* was higher in herbivore-damaged vs. herbivore-undamaged leaves, while *Pedobacter* spp. and *Pseudomonas fluorescens* infections were negatively associated with herbivory (Humphrey et al. 2014). All this evidence suggest that the composition and provenance of the microorganisms involved in the interaction between plants and insects should be identified before further experimental manipulation aimed at demonstrating their functional role.

The Gut Microbiota and Plant Defenses

In general, plant tissue consumption results in a reconfiguration of the primary and secondary metabolism. Several studies have reported that after damage, those processes related to the primary metabolism such as growth, photosynthesis, carbon assimilation, respiration, and reallocation of resources decrease (Zangerl et al. 2002; Schwachtje and Baldwin 2008), whereas the secondary metabolism, responsible for the production of chemical defenses, increases (Kessler and Baldwin 2002). These physiological and metabolic changes are closely related to the expression of defensive mechanisms of tolerance and resistance, respectively. It is now recognized that the expression of tolerance is related to changes in primary metabolism that allow tolerant genotypes to reduce the negative effects of herbivory in terms of fitness (Strauss and Agrawal 1999; Stowe et al. 2000; Fornoni 2011). On the other hand, increases in resistance are given by changes in the production and abundance of various secondary metabolites that prevent or limit the loss of foliar tissue (Fritz and Simms 1992; Karban and Baldwin 1997). The triggering of signaling cascades that produce changes in both metabolisms can be either initiated by endogenous biochemical pathways that start when plant cells are damaged (Mithöfer and Boland 2008) or initiated by elicitors of microbial origin present in the regurgitant of herbivorous insects (Felton et al. 2014).

The regurgitant of many insects contains chemical compounds with eliciting properties. Among the substances found in the regurgitant are plant growth promoters such as auxins (Dyer et al. 1995), pectinases (Hori 1975), indoleacetic acid (Miles and Lloyd 1967), epidermal growth factors (Detling and Dyer 1981; Dyer et al. 1995), cytokinins that increase the photosynthetic rate (Giron et al. 2007; Kaiser et al. 2010; Halitschke et al. 2011), and transcription factors involved in the transport of carbon and nitrogen (Steinbrenner et al. 2011) as well as in the reactivation of secondary meristems (Korpita et al. 2014). All these compounds have the potential to alter the tolerance response of plants through different mechanisms. Studies carried out with the tomato plant, *Solanum lycopersicum*, show that plants treated with *Manduca sexta* regurgitant recovered more quickly after a defoliation treatment by increasing their growth rate and the reactivation of secondary meristems (Korpita et al. 2014) probably because of metabolites involved in the transport of carbon and nitrogen that come in contact with the plant cells via the regurgitant (Steinbrenner et al. 2011). Some other studies have shown an effect of the regurgitant on plant traits, but the identity and origin of the particular elicitors are still unknown. For example, in the tobacco plant *Nicotiana attenuata*, it was found that damage by the moth *M. sexta* decreases the photosynthetic rate; however, when consumed by the hemipteran *Tupiocoris notatus*, a specific induction of elevated photosynthetic activity was shown (Halitschke et al. 2011). To our knowledge only one study has indeed shown that the gut endosymbionts are responsible for the production of these elicitors. Such is the case of the Lepidoptera *Phyllonorycter blancardella*, in which its endosymbionts such as *Wolbachia* spp. produce cytokinins that are deposited on the leaves, via regurgitation, leading to the formation of photosynthetically active “green patches” on damaged leaves (Kaiser et al. 2010). If a general pattern where the gut microbiota participates actively in the production of those compounds present in the regurgitant that triggers some tolerance-related trait, it is still to be confirmed.

On the other hand, there is also evidence that the regurgitant contains elicitors related to resistance mechanisms such as glucose oxidase (Diezel et al. 2009), polyphenol oxidases (Major and Constabel 2006; Ma et al. 2010), and proteinase inhibitors (Korth and Dixon 1997). The presence of such elicitors can sometimes decrease

(Bede et al. 2006; Lawrence et al. 2008; Weech et al. 2008; Chung and Felton 2011; Chung et al. 2013) or increase plant resistance (Spiteller et al. 2000; Musser et al. 2002; Ping et al. 2007; Diezel et al. 2009). Many studies have even identified the microbial symbionts known to be responsible for affecting plant resistance. The bacterium *Hamiltonella defensa* is a facultative endosymbiont of the whitefly *Bemisia tabaci*, and it is involved in the suppression of JA in tomato plants (Su et al. 2015). Also, in the tomato plant, the larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, exploit bacteria in their oral secretions to decrease the production of JA and JA-responsive antiherbivore defenses (Chung et al. 2013). In the same study system, applying bacteria isolated from larval oral secretions to wounded plants confirmed that three microbial symbionts belonging to the genera *Stenotrophomonas*, *Pseudomonas*, and *Enterobacter* were responsible for defense suppression (Chung et al. 2013).

Interestingly, the effects of the bacterial symbionts on plant resistance seem to be host-dependent. For example, bacterial isolates from oral secretions of the false potato beetle *Leptinotarsa juncta* belonging to the genera *Pantoea*, *Acinetobacter*, *Enterobacter*, and *Serratia* were found to suppress polyphenol oxidase activity in the non-preferred host tomato, while only *Pantoea* sp. was observed to suppress the same activity in the preferred host horsenettle (Wang et al. 2016). There is even evidence of potential trade-offs among resistance traits mediated by bacterial symbionts. The bacterium *Pantoea ananatis*, isolated from the oral secretions of the armyworm *Spodoptera frugiperda*, downregulates the activity of the proteins polyphenol oxidase and trypsin proteinase inhibitors, but upregulates the peroxidase activity in the tomato plant (Acevedo et al. 2017). In turn, plant chemical defense can also affect the composition and structure of the insect microbial community. In the trembling aspen, *Populus tremuloides*, phenolic glycosides and condensed tannins affected the relative abundances of *Ralstonia* and *Acinetobacter* in the midgut of the gypsy moth *Lymantria dispar* (Mason et al. 2015). Taken together, these examples show the potential feedbacks between insect microbial communities and plant resistance. However, future studies should be designed to specifically test reciprocal feedbacks between the gut microbiota and plant resistance. Overall, all the evidence points at microorganisms modulating the expression of tolerance and resistance mechanisms against herbivory. We visualized two approaches that can complement each other to disentangle the evolutionary role of plant and insect microbiotas: network theory and experimental studies.

Network Theory and Interactions Among Microbiotas

Over the last 20 years, it has been recognized the value and importance of networks in a myriad of applications in biology. The concepts and tools developed from graph theory have provided new insights into evolutionary ecology as well as a valuable conceptual framework to address new challenges. Fundamental concepts from ecological systems – such as communities – to networks of biological interactions among their components provide a way to summarize large amounts of information within single objects. Perhaps, one of the most successful examples of the application of network theory in biology is plant science where key regulators, functional modules, and novel phenotypes have been identified through gene regulatory networks (Álvarez-Buylla et al. 2007). In the field of microbial evolution, network theory has been used to identify new targets for probiotic treatments (Lemon et al. 2012), find the most influential biotic and abiotic factors that structurally change the structure of the microbial community (Fisher and Mehta 2014), and distinguish the topological properties of microbial networks between health and disease states (Sánchez et al. 2017; Sommer et al. 2017). Microbial networks thus constitute a heuristic tool that could help us model and understand the complexity of the interactions within the microbiota and among microbiotas of interacting hosts.

In general, ecological networks can be divided in static or dynamic networks. Static networks focus on the study of its topological properties: measures of centrality (the relative importance of the nodes, each node representing a particular microorganism), the distribution of links to other nodes (degree distribution), identifying modules or clusters, and finding overrepresented or recurrent subgraphs (motifs). These kinds of networks are useful because they take into account any binary dependence between the elements, that is, the presence-absence of interactions among species. Weighted networks are a generalization of static networks, adding to the edges a measure of the relevance or certainty of its links. This value can be represented by the frequency or the strength of the different interactions. On the other hand, dynamical or evolving networks represent not only the species (i.e., microorganisms) and the topology/strength of the interactions but also the dynamic nature of the whole system (i.e., the holobiont). However, these kinds of networks are more difficult to infer from empirical data because they need a detailed and specific information almost never available. Due to the fact that static networks are much easier to obtain from data, the ample majority of ecological networks belong to this kind.

In microbiome studies, particular approaches have been adapted and combined to infer co-ocurrence networks of OTUs, interaction networks, and a new multi-network approach involving networks of microorganisms with its host. In a seminal paper, Gould and collaborators (2018) mapped the interactions between individual species of bacteria against several fitness traits of its hosts, the fruit fly. They showed that the same bacterial interactions that shape microbial abundances in the microbiota also determine the fly fecundity. In a recent study, Huitzil et al. (2018) proposed an evolutionary computational model in which a network representing the host can adapt in order to perform a predefined function related to its host. In this model, the host network interacts with its microbial network, and these complex interactions can explain the presence of dysbiosis, specialization, and microbial diversity.

One of the most important goals in microbial networks is to identify the so-called keystone taxa in microbial communities and to determine the factors that influence its function in a given environment. The dominant taxa could be the most abundant or the most important, structurally speaking, in terms of the topology of the network. In an extraordinary example, Flores et al. (2013) observed that complex networks between host and parasites or between bacteria and phages are at the same time, but at different scales, modular and nested. This observation suggests that different evolutionary regimes operate at different scales. The next natural step would be to integrate various layers of information via multiple single networks. This approach is called multilayer networks or multi-networks and could be defined as an amalgamation of networks that interact and evolve with each other (Bianconi 2018). Multilayer network applied to whole holobionts and to the interactions among holobionts could be an extremely valuable tool in understanding microbe-insect-plant interactions. Each one of these networks (plant microbiota network and insect microbiota network) will then form a meta-network, which will undoubtedly be a more realistic approach to the study of ecological interactions among holobionts. Thus, multilayer networks have the potential to take into account multitrophic interactions (Mirzaei and Maurice 2017).

Experimental Studies

To date most studies about microbe-insect-plant interactions have concentrated on the description of the microbiotas within multicellular species as well as in the sources of possible environmental regulation. One of the major assumptions behind these studies is that microorganisms directly affect the survival, performance, and fitness of their hosts as well as the interactions between hosts and other community members. Correlative evidence support this premise but have not yet identified the functional role of most microbial lineages. If microorganisms have the potential to regulate the ecological and evolutionary dynamics between plants and insects, then our technological efforts to reduce or control herbivory levels should not neglect the effects of microorganisms. Pests are usually controlled with the use of insecticides and/or genetically modified crops, whereas the manipulation of microorganisms within plants or insects is still underdeveloped. We highlight two experimental approximations that can shed light into the functional role of microorganisms within the context of a plant-insect interaction. First, performing cross-infection experiments (i.e., reciprocal transplants) of microorganisms from different host populations or environmental conditions can provide relevant information on their role for plant and insect coadaptation to each other. These kinds of experiments are also fundamental to differentiate the relative importance of natural selection and genetic drift in the conformation and function of the microbial communities. Second, experiments where the microbiota composition is manipulated, either the presence/absence or the relative abundance of certain microorganisms, can identify their functional role on the plant-insect interaction. These experimental approximations, among others, will demonstrate whether the understanding and manipulation of plants and insects require the recognition that the evolution of the interaction depends not only upon the plant and insect genetics within a specific environmental context but also on the microbiome as well.

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Chapter 8

Ecosystem Engineering by Insect Herbivores: Non-trophic Interactions in Terrestrial Ecosystems

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Abstract Ecosystem engineering is recognized as a relevant non-trophic interaction with overall positive effects on biodiversity. Ecosystem engineers (organisms that modify or create new habitats) are distributed in all type of ecosystems, but they are particularly abundant among insect herbivores. Ecosystem engineering by insect herbivores occurs as the result of structural modification of plants, which can involve specialized adaptations to manipulate the tissues and physiology of their host plants. Recent research suggests that these adaptations could play an important role in the evolution of plant-insect interactions and insect diversification. In this chapter, I present a review of ecosystem engineering by insect herbivores, discussing the diversity and evolutionary origins of the main insect herbivore groups acting as ecosystem engineers, with a special focus on endophagy and plant manipulation strategies that enable insects to create new habitats. I also discuss the mechanisms and effects of habitat modification on arthropod communities associated with the modified habitats, as well as the evolutionary consequences of habitat modification. A qualitative review of the arthropod community associated with habitats engineered by different guilds of insect herbivores is presented. Finally, future directions and perspectives regarding key questions for further research are identified.

Keywords Habitat modification · Shelter construction · Plant manipulation · Ecological facilitation · Bottom-up effects · Top-down effects · Trophic cascades · Niche construction · Endophytic insects · Arthropod diversity

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Non-trophic facilitative interactions, such as ecosystem engineering, are increasingly recognized as relevant ecological and evolutionary drivers of diversity (Jones et al. 2010; Matthews et al. 2014; Sanders et al. 2014; Romero et al. 2015; Cornelissen et al. 2016).

Ecosystem engineering is the process of habitat modification, by which some organisms (i.e., ecosystem engineers) directly or indirectly control resource availability for other organisms through physical, chemical, or structural changes in the biotic and abiotic components of their habitat (Jones et al. 1994, 1997; Lawton and Jones 1995).

The concept of ecosystem engineering was introduced in 1993 at the Fifth Cary Meeting at the Institute of Ecosystem Studies, Millbrook, New York (Lawton and Jones 1993), and formally described by Jones et al. (1994). However, the process itself was first mentioned by Darwin in his book *The Formation of Vegetable Mould Through the Action of Worms* (1881), in which he discussed how earthworms modify their soil habitat by their own activities in a feedback process that results in earthworm adaptation. In addition to bioturbation (i.e., changes in soil properties caused by the activity of organisms), diverse activities that involve the addition, removal, reconfiguration, and redistribution of resources in the habitat (e.g., light, salinity, nutrients, wood cavities, nesting sites, substrates for attachment) are also recognized as ecosystem engineering processes (Wright and Jones 2004; Jones et al. 2010).

After the introduction of the concept, different theoretical models predicting ecosystem engineering effects were developed (e.g., Cuddington and Hastings 2004; Wright and Jones 2004; Hastings et al. 2007; Cuddington et al. 2009; Jones et al. 2010; Raynaud et al. 2013; Sanders et al. 2014; Romic and Nakajima 2018), and numerous empirical studies have been conducted (reviewed in Wright and Jones 2004; Romero et al. 2015; Cornelissen et al. 2016). Predictions based on these models indicate that the impacts of ecosystem engineering can vary spatially and temporarily depending on several factors that include the nature of the structural change, habitat productivity, the engineer population, resource availability, the time of persistence of the modified habitat, and the number of species in the regional pool that use the resources; however, empirical evidence indicates that overall effects on diversity are positive. This is consistent with the results of a recent meta-analysis of 122 studies reporting that (i) ecosystem engineering has a higher effect on species richness in tropical ecosystems (83%) than in ecosystems at higher latitudes (15%); (ii) engineers that create habitats in terrestrial ecosystems increased the species richness by 300%, whereas engineers that modify habitats such as bioturbators had no significant effect on species richness (even though they increase productivity); (iii) invertebrate engineers had stronger effects on species richness than vertebrate engineers; and (iv) within aquatic habitats, engineering effects are stronger in marine ecosystems than in freshwater streams, whereas in terrestrial habitats, arid ecosystems exhibit the strongest effects (Romero et al. 2015). Interestingly, most of the organisms with the strongest effects on species richness included in this meta-analysis are insect herbivores, indicating that ecosystem engineering is an ecologically important interaction that has a key role in biodiversity (Fukui 2001; Lill and Marquis 2007; Cornelissen et al. 2016; Calderón-Cortés et al. 2016).

Ecosystem Engineering by Insect Herbivores

Several studies have demonstrated that insect herbivores act as ecosystem engineers through the physical and structural modifications of plants manipulated by insects (e.g., Martinsen et al. 2000; Johnson et al. 2002; Lill and Marquis 2003; Lill et al. 2007; Nakamura et al. 2003; Kagata and Ohgushi 2004; Crawford et al. 2007; Marquis and Lill 2007, 2010; Utsumi and Ohgushi 2009; Calderón-Cortés et al. 2011, 2016; Wang et al. 2012; Yoneya et al. 2014; Novais et al. 2017, 2018). Physical and structural modifications of plants by insect herbivores usually occur as the result of feeding (Calderón-Cortés et al. 2016) and by the construction of shelters (Fukui 2001; Lill and Marquis 2007). These structures or shelters as well as the resources related to plant modifications are potentially available for other organisms that can co-occupy the shelters or occupy them after abandonment by the engineer species. Common guilds of herbivores that act as ecosystem engineers include leaf shelter builders, leaf miners, gall inducers, and stem borers (Marquis and Lill 2007; Cornelissen et al. 2016; Calderón-Cortés et al. 2016) (Fig. 8.1).

Leaf Shelter Builders

Many larval insects construct simple external structures by manipulating a portion of a leaflet, an entire leaf, or multiple leaves with silk as they grow (Cornelissen et al. 2016). These structures can take the form of rolls, webs, ties, folds, or tents depending on how the insects manipulate the leaves (Lill et al. 2007; Lill and Marquis 2007). For example, leaf rollers and leaf folders use entire leaves or part of a leaf to roll/fold them with silken threads, whereas leaf-tiers and leaf-tent builders bind two or more leaves or a flap of leaf previously cut with silk strands (Lill and Marquis 2007; Cornelissen et al. 2016) (Fig. 8.1d–h). Most leaf shelter builders feed inside the constructs, but others use the leaf shelters for protection (Fukui 2001; Lill and Marquis 2007; Marquis and Lill 2010).

Leaf Miners

Leaf miners are mobile consumers that feed internally on soft live foliar tissue located between the upper and lower epidermal layers of leaves without disrupting the leaf surfaces, thereby creating cavities or channels known as mines (Fig. 8.1i–k; Hering 1951; Connor and Taverner 1997). Mines can be classified as follows: (i)

Fig. 8.1 Structural modifications of plants carried out by insect herbivores acting as ecosystem engineers. (**a**): leaf galls, (**b**): stem galls, (**c**): bud galls, (**d**–**g**): leaf shelters, (**h**–**j**): leaf mines, (**k**): galleries made by stem borer beetle larvae, (**l**–**m**): stem borer exit holes in branches. (Credit photo: N. Calderon (**a**–**c, g**–**m**), E. del Val (**d**–**e**))

linear mines with different shapes, including serpentine, spiral, and branched mines, which indicate that the miner keeps feeding forward; (ii) blotch mines with circular, oval, rectangular, tentiform, or amoeboid shapes, which indicate that the miner feeds in several directions or in a combination of directions; and (iii) linear-blotch mines, with a series of transitional mine shapes (Hering 1951; Liu et al. 2015).

The construction of a mine represents a distinctive specialization of an endophagous lifestyle (Hering 1951; Connor and Taverner 1997) that begins with the oviposition of an egg in the leaf tissue, followed by larval consumption, and usually ends in an enlarged terminal chamber used for pupation (Labandeira 2002). However, some leaf miners can move among leaves (i.e., mine abandonment followed by the initiation of a new mine) before pupation, and mining can be limited to a single larval instar in others (Hering 1951). After pupation, the insect emerges from the mine by making a hole in the foliar epidermis, leaving a vacant mine that can be used by other arthropods (Kagata and Ohgushi 2004).

Gall Inducers

Insect galls are atypical structures (new organs) composed of plant tissues that develop as a response to stimuli produced by a gall inducer (Stone and Schönrogge 2003; Price 2005). Galls range in complexity from relatively open pits or folds to structures in which the gall inducer is entirely enclosed by the plant tissues (Stone and Schönrogge 2003). Such enclosed galls range from simple to highly differentiated structures, comprising different layers of nutritive inner tissues and complex protective outer tissues (Stone and Schönrogge 2003; Giron et al. 2016). Galls usually occur on leaves and stems, but they may also occur on any vegetative or reproductive plant tissues (Fig. 8.1a–c).

Gall inducers include insects, mites, nematodes, fungi, bacteria, and viruses (Stone and Schönrogge 2003; Giron et al. 2016). In insects, the mechanisms underlying gall induction and development are largely unresolved, but they usually involve a fast differentiation of new nutritive cells by a combination of cell division (i.e., hyperplasia) and growth (i.e., hypertrophy), an increase in cellular organelles, fragmentation of vacuoles, endoreduplication of nuclei, and enlargement of plasmodesmata to allow communication between the cell layer and vascular tissues (Fernandes et al. 2012; Oliveira et al. 2016). Hence, the resulting galled tissue provides to the gall inducer a resource that is higher in nutritional quality than non-galled tissue (Price 2005). However, galls can also be colonized by diverse organisms: inquilines that use the original gall to build new one, parasitic inquilines that consume the gall and parasitized the gall inducer, fungal pathogens, and secondary users that colonize the gall after the emergence of the gall inducers (Hayward and Stone 2005; Marquis and Lill 2007; Cornelissen et al. 2016).

Stem Borers

Stem borers are endophagous insects that develop in wood, bark, or woody stems of plants during at least part of their life cycle. Many of them begin their life cycle as eggs laid under bark by free-living adult females, but the larvae feed on the wood inside the stems and eventually emerge as adults to repeat the cycle (Lieutier et al. 2004). The stem-boring larvae possess mandibulated mouthparts that allow them to tunnel inside plant tissues (especially wood but also bark, collenchyma, and sclerenchyma) and produce complex systems of cavities (Fig. 8.1l–m) that can be secondarily occupied by other arthropods (Labandeira 2002; Marquis and Lill 2007; Calderón-Cortés et al. 2011). Some girdling borers can also increase nutrient content in the tissue in which they develop by interrupting phloem flow (Forcella 1982; Hanks 1999; Calderón-Cortés et al. 2016).

Diversity of Herbivorous Ecosystem Engineers

Leaf shelter building habits are present in several insect orders, such as Orthoptera, Hemiptera, Thysanoptera, Hymenoptera, Coleoptera, and Diptera, although this feeding habit is widespread in Lepidoptera in at least 24 families (Fukui 2001; Lill and Marquis 2007). The Lepidoptera families that commonly build leaf shelters are Hesperiidae, Gelechiidae, Oecophoridae, Tortricidae, Elachistidae (Stenomatinae), Pyralidae, Choreutidae, Crambidae, Lasiocampidae, and Thyrididae (Marquis and Lill et al. 2007; Lill and Marquis 2007). Other families, such as Nymphalidae, Notodontidae, Noctuidae, Erebidae (Arctiinae), Plutellidae, Geometridae, and Pamphiliidae, also include some species that build leaf shelters (Lill and Marquis 2007). Leaf shelter-building insects are important components of the herbivore community in several regions and ecosystems because they constitute 20–25% of the total lepidopteran fauna (Lill and Marquis 2007; Cornelissen et al. 2016).

Leaf miners comprise approximately 10,000 species of 51 families belonging to the four major holometabolous insect orders: Coleoptera, Hymenoptera, Diptera, and Lepidoptera (Connor and Taverner 1997; Labandeira 2002; Cornelissen et al. 2016), but it is suspected that more than 90% of leaf-mining species remain undiscovered (Cornelissen et al. 2016; Liu et al. 2015). Among these insect orders, the leaf-mining habit is most widely distributed in Lepidoptera (34 families), mostly in dytrisian families and some monotrysian families (for details, see Connor and Taverner 1997). Leaf mining is also present in nine dipteran families (mainly Agromyzidae and Ephydridae), six coleopteran families (mainly Chrysomelidae), and three hymenopteran families belonging to the superfamily Tenthredinoidea (Connor and Taverner 1997).

In terms of gall inducers, 13,000 insect species are described, but estimates of global species richness based on the number of gall inducers per plant species range between 11,000 and 211,000, with an average of 133,000 species, indicating that only 2% of the total species richness is known (Raman et al. 2005; Espirito-Santo and Fernandes 2007). The ability to induce galls is present in at least 51 families distributed in 6 different orders: Hemiptera, Thysanoptera, Coleoptera, Hymenoptera (Cynipidae, Eurytomidae, and Tenthredinidae), Lepidoptera, and Diptera (Cecidomyiidae and Tephritidae), but the highest diversity is found in Diptera (mainly Cecydomyiidae and Tephritidae), followed by Hymenoptera (Cynipidae and Eurytomidae) (Fernandes et al. 2012).

Insect stem borers are predominantly beetles (Coleoptera) of seven families, Cerambycidae, Buprestidae, Curculionidae, Bostrichidae, Anobiidae, Lymexylidae, and Cupedidae, but also include some species of two Hymenoptera families

(Siricidae and Xiphydriidae) and six Lepidoptera families (Cossidae, Sesiidae, Pyralidae, Hepialidae, Noctuidae, and Tortricidae) (Haack and Slansky 1987; Grehan 1987; Lieutier et al. 2004; Labandeira 2002; Chiappini and Aldini 2011). Nevertheless, lepidopterans are cambium or pith borers that consume soft tissues, and they alternate between external feeding and boring during their life cycle (Grehan 1987; Labandeira 2002). The three main beetle families that include stem borers comprise ~56,000 species (35,000, 15,000, and 6000 for Cerambycidae, Buprestidae, and Scolytinae, respectively), representing 12–15% of the described coleopteran species (Hanks 1999; Evans et al. 2007; Kirkendall et al. 2015).

The diversity of insects with the ability to modify their habitat indicates that Holometabola concentrates the highest species richness of ecosystem engineers in four orders, Coleoptera, Hymenoptera, Lepidoptera, and Diptera (Fig. 8.2a), which correspond with the main superradiations of insect herbivores and the Cretaceous rise of angiosperms, in addition to Hemiptera (Trautwein et al. 2012). Coleoptera, Hymenoptera, and Lepidoptera include the four feeding habits found in ecosystem engineers: stem boring, leaf mining, leaf shelter building, and gall inducing; meanwhile, Diptera includes gall inducers, leaf miners, and leaf shelter builders; and

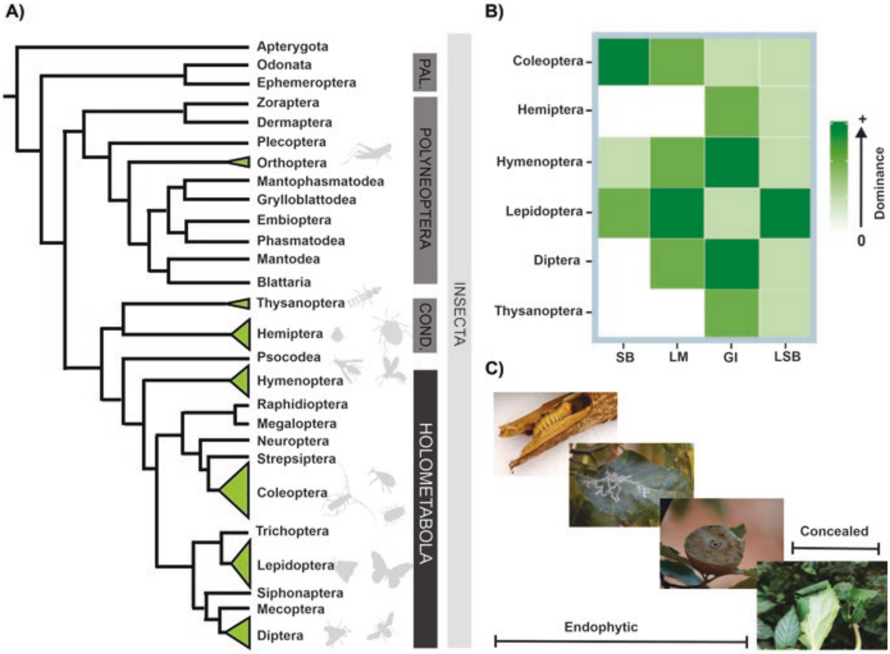

Fig. 8.2 Diversity of herbivorous ecosystem engineers. (**a**) Insect orders including species acting as ecosystem engineers are shown in a phylogeny drawn based on Trautwein et al. 2012. (**b**) Relative dominance of each ecosystem engineering feeding guild in different insect orders. *SB* stem borers, *LM* leaf miners, *GI* gall inducers, *LSB* leaf shelter builders. (**c**) Classification of feeding guilds according to lifestyle: endophytic insects include stem borers, leaf miners, and gall inducers from left to right; concealed insects include leaf shelter builders

Hemiptera and Thysanoptera only include gall inducers and leaf shelter builders (Fig. 8.2b). This suggests that the evolution of ecosystem engineers within the different insect orders is complex and involves several independent origins. In accordance with this, previous studies have reported that the evolution of feeding habits in different insect orders implies multiple origins and shifts (Connor and Taverner 1997; Crespi et al. 1997; Cook and Gullan 2004; Farrell and Sequeira 2004; Labandeira 2002; Regier et al. 2012; Mitter et al. 2017). For example, leaf mining is recognized as the ancestral feeding habit in Lepidoptera, while stem boring and leaf shelter building represent derived traits that originated independently in different families of this insect group (Connor and Taverner 1997; Mitter et al. 2017). In contrast, leaf mining is a derived trait in Coleoptera, as well as leaf shelter building and gall induction, which have arisen several times from an external feeding habit, even though Cupedidae, the most ancestral beetles, are stem borers (Farrell and Sequeira 2004; Grimaldi and Engel 2005; Hunt et al. 2007). In the case of Hymenoptera, the three feeding modes among the three guilds of ecosystem engineers reported (stem boring, leaf mining, and gall inducing) are derived traits that arose from external feeding (Heraty et al. 2011).

Previous studies suggest that some feeding shifts were followed by adaptive radiation events, as occurred in gall inducers (sawflies, gall midges, aphids, and oak cynipid wasps; reviewed in Price 2005) and likely in Cerambycidae (i.e., Lamiinae) stem borers (Farrell and Sequeira 2004), explaining to some extent the differences in the dominance of feeding guilds among insect orders (Fig. 8.2b). Interestingly, several transitions from an external feeding habit to an endophytic or concealed feeding habit (Fig. 8.2c), as well as transitions from one endophytic to another endophytic feeding habit (i.e., leaf mining to gall inducing, leaf folding and rolling to leaf galling; Price et al. 1987; Nyman et al. 2000), suggest that endophagy played a key role in the origin and diversification of insect ecosystem engineers (Price et al. 1987; Stone and Schönrogge 2003; Price 2005), as has been previously suggested (Giron and Huguet 2011; Giron et al. 2016).

Adaptive Nature and Evolution of Habitat Modification: What Role Do Endophagy and Plant Manipulation Play?

Three major non-mutually exclusive hypotheses have been proposed to explain the adaptive nature of endophagy and shelter-building behavior in insect herbivores: the microenvironment hypothesis, the enemy hypothesis, and the nutritional hypothesis (Price et al. 1987; Stone and Schönrogge 2003; Lill and Marquis 2003; Price 2005).

The microenvironment hypothesis postulates that endophagy and plant shelters constructed by ecosystem engineers provide protection to the shelter-building insects against environmental conditions such as extreme temperatures, low humidity, solar radiation, and wind/rain exposure (Fukui et al. 2002; Lill and Marquis 2007). Evidence supporting the microenvironment hypothesis has been reported for gall inducers, leaf rollers, leaf tiers, and leaf miners, but specific benefits differ among feeding guilds. Protection from desiccation (i.e., hygrothermal stress) appears to be most important for gall inducers (Price et al. 1987; Fernandes and Price 1992). High relative humidity helps to maintain constant osmolarity of the hemolymph in some leaf roller caterpillars, and it is also related to an increase in growth, survival, or adult mass in some species (reviewed in Lill and Marquis 2007). Protection from UV radiation and more favorable temperatures (particularly in cold areas) are reported for leaf miners (Connor and Taverner 1997).

On the other hand, the enemy hypothesis proposes that endophytic and/or concealed feeding in plant shelters confers protection against natural enemies including predators, parasitoids, and pathogens (Price et al. 1987). Empirical evaluations indicate higher or equal levels of parasitism in endophytic/concealed than in external feeders and highly diverse parasitoid communities in plant shelters, suggesting that shelter-building behavior does not provide protection against parasitoids (Price et al. 1987; Connor and Taverner 1997; Stone and Schönrogge 2003; Hayward and Stone 2005; Lill and Marquis 2007). However, at least for gall inducers, an initial period of enemy escape cannot be excluded (i.e., ghost of past parasitism), since galls could provide protection against parasitoid attack before the parasitoids evolve the ability to exploit them (Price et al. 1987; Hayward and Stone 2005). This also applies to some predators initially excluded from plant shelters (by avoiding visual detection and/or by excreting toxic repellent compounds in feces: Fukui 2001; Lill and Marquis 2007) that have evolved the ability to use plant shelters as visual cues (i.e., bird learning: Murakami 1999) or volatiles in feces to locate their preys (Lill and Marquis 2007), suggesting the rapid adaptation of predators to circumvent the protective traits of their prey without excluding initial protection.

Finally, the nutrition hypothesis states that shelter builders have enhanced access to high-quality food resources by feeding on the most nutritious tissues and/or avoiding major physical and chemical plant defenses (Connor and Taverner 1997; Fukui 2001; Stone and Schönrogge 2003; Lill and Marquis 2007; Giron et al. 2016). Available evidence indicates that leaf shelter builders (i.e., leaf rollers and leaf tiers) can improve the quality of the manipulated plant tissues as a response to reduced exposure to light, which results in decreased leaf toughness and lower concentrations of tannins, phenolics, and other antiherbivore defense compounds (i.e., lignin, hypericin, and other phototoxic compounds) in leaf shelters (Fukui 2001; Lill and Marquis 2007). However, there is no evidence showing that leaf shelters have higher nutritional quality than leaves without shelters (reviewed in Lill and Marquis 2007).

On the other hand, endophytic insects such as leaf miners and stem borers feed selectively on the most nutritious layers, restricting their feeding to tissue layers with low concentrations of chemical defenses and without some physical defenses (i.e., spines and trichomes restricted to plant surfaces) to avoid plant defenses (Connor and Taverner 1997; Haack and Slansky 1987; Hanks 1999). Some of these insects can also manipulate the nutritional quality of their host plant tissues by disrupting the vasculature (i.e., leaf miners that damage the leaf midrib and stem girdler-borers: Johnson et al. 2002; Forcella 1982; Hanks 1999; Calderón-Cortés et al. 2011, 2016). Consequently, through the structural manipulation of the host plant, these insects block the downward translocation of photosynthates and metabolites through the phloem, increase the nutrient content in tissues adjacent to the site of damage, and avoid plant defenses (Li et al. 2003; Forcella 1982; Andersen et al. 2002; Johnson et al. 2002; Ganong et al. 2012; Calderón-Cortés et al. 2016). Similarly, gall inducers (one of the most emblematic plant reprogrammers) and leaf miners that induce the production of "green islands" can also actively manipulate the quality of their host plants. The mechanisms underlying the active manipulation of plant physiology by these insects remain unknown and might involve complex interactions with symbiotic microorganisms, but they commonly result in the development of nutritive cells (galls) or photosynthetically active cells in otherwise senescent tissues (green islands), upregulation of protein and sugar synthesis, translocation of nutrients, and/or modulation of plant secondary metabolism (i.e., by diminishing the phenolic and tannin concentration at the feeding site) to circumvent plant defenses (Stone and Schönrogge 2003; Giron et al. 2007; Kaiser et al. 2010; Giron and Huguet 2011; Body et al. 2013; Giron et al. 2016; Oliveira et al. 2016).

Interestingly, the existence of different strategies to improve nutritional quality (i.e., selective feeding, structural manipulation, and active manipulation of plant physiology) in endophytic insects not observed in leaf shelter builders (concealed insects) suggests that nutritional constraints imposed by an endophytic lifestyle (Giron and Huguet 2011; Giron et al. 2016) could lead to the evolution of different adaptations in insect herbivores that allow them to use relatively more nutritious and less defended tissues and to increase the availability of nutrients in the manipulated plant tissues, explaining the effects that these insects can have on other organisms using the modified habitats.

Mechanisms and Effects of Habitat Modification by Insect Herbivores

Several experimental studies have reported that habitat modification (i.e., through the construction of plant structures) by insect herbivores has strong effects on the abundance and species richness of arthropod communities (Martinsen et al. 2000; Johnson et al. 2002; Bailey and Whitham 2003; Lill and Marquis 2003, 2004; Nakamura et al. 2003; Kagata and Ohgushi 2004; Crawford et al. 2007; Marquis and Lill 2007; Utsumi and Ohgushi 2009; Calderón-Cortés et al. 2011; Vieira and Romero 2013;Yoneya et al. 2014; Uesugi et al. 2016; Novais et al. 2017, 2018; Henriques et al. 2018; Santos et al. 2019). Indeed, a recent meta-analysis of experimental studies evaluating the effects of ecosystem engineering by insect herbivores showed that the structural modification of plants increased arthropod richness by 128% and abundance by 135% in local communities (Cornelissen et al. 2016). This indicates that herbivorous ecosystem engineers provide resources that are readily exploited by a great number of secondary users.

Table 8.1 Arthropod community using plant habitats created by herbivorous ecosystem engineers

Taxa (Class/ Order)	Leaf shelter builders	Leaf miners	Stem borers	Gall inducers
Arachnida				
Acari	Trombiculidae (1)		Not identified	Acaridae
				Oribatida
				Phytoseiidae
Araneae	Anyphaenidae (4)^P	Thomisidae (1)	Not identified (41)^P	Araneidae
	Araneidae (11)^P	Tetragnathidae (1)		Clubionidae (1)^P
	Corinnidae (3)^P	Clubionidae (1)		Linyphiidae
	Linyphiidae (3)^P			Philodromidae
	Mimetidae (1)^P			Salticidae (1)^P
	Miturgidae (2)^P			Theridiidae
	Oxyopidae (1)^P			
	Pisauridae (1)^P			
	Salticidae (7)^P			
	Scytodidae (1)^P			
	Segestriidae (1)^P			
	Sparassidae (2)^P			
	Tetragnathidae (3) ^P			
	Theridiidae (11)^P			
	Thomisidae (2)^P			
Opiliones	Gonyleptidae (1)^P		Not identified (1) ^P	
Pseudoscorpiones	Not identified (1)^P		Not identified (4)^P	
Solifugae			Not identified (1)^P	
Chilopoda				
			Geophilomorpha (6)^P	
Collembola				
	Entomobryidae (2)^D	Entomobryidae (2)	Not identified	Entomobryidae
	Hypogastruridae (1)^D			
	Isotomidae (2)^D			
	Sminthuridae (1)^D			
	Tomoceridae (1)^D			
	Not identified			
Diplopoda				
	Not identified^D		Spirobolida (2)	Polyxenidae (1)
Insecta				
Blattodea	Blattidae^D		Not identified (3)	Rhinotermitidae (1)

(continued)

Table 8.1 (continued)

Taxa (Class/ Order)	Leaf shelter builders	Leaf miners	Stem borers	Gall inducers
Coleoptera	Aderidae (1)^{D}		Anthicidae (2)^{P}	Anobiidae (3)
	Anthicidae (1)^{P}		Carabidae (3)^{P}	Buprestidae
	Biphyllidae (1)^{D}		Bostrichidae (14)^{B}	Bruchinae (3)
	Buprestidae (1)^{H}		Buprestidae (16)^{B}	Cerambycidae (>2)
	Cantharidae (2)^{P}		Cantharidae (1)^{P}	Cleridae (5) ^{P}
	Carabidae (1)^{P}		Cerambycidae (81)^{B}	Coccinellidae (1)^{N}
	Chrysomelidae (12)^{H}		Cleridae (8)^{P}	Curculionidae
	Coccinellidae (6)$^{H, P}$		Cryptophagidae (1)	Latridiidae (1)
	Corylophidae (2)^{D}		Cucujidae (1)	
	Curculionidae (13)^{H}		Curculionidae (5)	
	Elateridae (3)^{H}		Elateridae (1)	
	Lycidae (1)^{D}		Histeridae (1)	
	Mordellidae (1)^{H}		Laemophloeidae (1)^{P}	
	Nitidulidae (2)^{H}		Mordellidae (1)	
	Ptilodactylidae (1)^{D}		Silvanidae (1)	
	Staphylinidae (16)^{P}		Staphylinidae (1)^{P}	
	Tenebrionidae (1)^{H}		Tenebrionidae (3)	
			Trogossitidae (3)	
Dermaptera	Forficulidae (2)			Forficulidae (1)
Diptera	Anthomyiidae (1)^{S}		Not identified^{S}	Cecidomyiidae (2)
	Cecydomyiidae (2)^{S}			Muscidae (3)^{N}
	Ceratopogonidae (1)^{S}			
	Chloropidae (1)^{D}			
	Drosophilidae (1)^{D}			
	Empididae (1)^{P}			
	Phoridae (2)^{S}			
	Sciaridae (4)^{D}			
	Stratiomyidae (1)^{P}			
	Not identified			
Hemiptera	Acanthosomatidae (2)	Aphididae (1)	Coreidae (1)	Aphididae (1)
	Achilidae (1)^{H}		Pentatomidae (1)	
	Aleyrodidae (1)^{H}			

(continued)

Table 8.1 (continued)

Taxa (Class/ Order)	Leaf shelter builders	Leaf miners	Stem borers	Gall inducers
	Anthocoridae (1)			
	Aphididae (7)^{H}			
	Cercopidae (1)^{H}			
	Cicadellidae (22)^{H}			
	Cixiidae (1)			
	Coccidae (4)^{H}			
	Diaspididae (1)			
	Dictyopharidae (1)^{H}			
	Isotomidae (2)^{D}			
	Lygaeidae (1)^{H}			
	Membracidae (4)^{H}			
	Miridae (6)^{H}			
	Pemphigidae (3)			
	Pentatomidae (2)			
	Phymatidae (1)^{P}			
	Reduviidae (2)^{H}			
	Scutelleridae (1)^{H}			
	Tingidae (1)^{H}			
Hymenoptera	Crabronidae (1)^{P}	Braconidae (3)^{S}	Braconidae (6)^{S}	Bethylidae (3)^{S}
	Diapriidae (2)^{S}	Chalcididae^{S}	Eupelmidae (1)^{S}	Braconidae (2)^{S}
	Encyrtidae (2)^{S}	Encyrtidae (2)^{S}	Eurytomidae (4)^{S}	Chrysididae (1)^{S}
	Eulophidae (2)^{S}	Eulophidae (32)^{S}	Formicidae (16)	Cynipidae (2)^{G}
	Figitidae (1)^{S}	Formicidae (1)^{N}	Pteromalidae (1)^{S}	Eulophidae (3)^{S}
	Formicidae (30)$^{N, O, P}$	Ichneumonidae^{S}	Not identified (6)^{S}	Eupelmidae (2)^{S}
	Mutillidae (1)^{S}			Eurytomidae (4)^{S}
	Platygastridae (1)^{S}			Formicidae (27)$^{P, N}$
	Pergidae (1)			Ichneumonidae (1)^{S}
	Scelionidae (1)^{S}			Ormyridae (1)
	Tenthredinidae (2)			Pteromalidae^{S}
	Tetragnathidae (1)^{P}			Sphecidae (2)$^{P, N}$
				Torymidae (2)^{S}
				Vespidae (3)$^{P, N}$
Lepidoptera	Arctiinae (6)^{H,I}	Crambidae	Tineidae	Gelechiidae (1)
	Bucculatricidae (2)^{I}	Gelechiidae		Pyralidae (1)
	Gelechiidae (14)^{L,I}	Oecophoridae		Sesiidae (1)
	Geometridae (11)$^{H, I}$	Pyralidae		Tortricidae (1)
	Gracillariidae (2)^{I}	Tortricidae		
	Hesperiidae (3)^{L}	Yponomeutidae		
	Limacodidae (7)^{I}			

(continued)

Table 8.1 (continued)

Taxa (Class/ Order)	Leaf shelter builders	Leaf miners	Stem borers	Gall inducers
	Lymantriidae (2)^{I}			
	Nepticulidae (1)^{I}			
	Nymphalidae (3)^{H}			
	Noctuidae (8)^{L,I}			
	Notodontidae (4)^{I}			
	Oecophoridae (8)^{L,I}			
	Pyralidae (10)^{L}			
	Psyllidae (1)^{H}			
	Saturniidae (1)^{H}			
	Stenomatinae (4)			
	Tischeriidae (2)^{I}			
	Tortricidae (5)^{L,I}			
	Not identified(15)^{H}			
Neuroptera	Chrysopidae (1)			Chrysopidae (2)
Orthoptera	Acrididae (3)^{O}		Gryllidae (6)	
	Gryllidae (3)^{O}			
	Phalangopsidae(2)^{O}			
	Tettigoniidae (4) ^{O}			
Phasmatodea	Heteronemiidae (2)			
	Not identified (1)^{H}			
Psocoptera	Amphipsocidae (1)^{D}			Not identified (2)
	Pseudocaeciliidae (2)^{D}			
	Psocidae (1)^{D}			
Thysanoptera	Phlaeothripidae (1)^{H}	Not identified		
	Thripidae (2)^{H}			
Malacostraca				
Isopoda	Trachelipodidae (1)^{D}		Not identified (1)	
	Not identified (1)			

The approximate number of species for each insect family is reported in parentheses. ^{B} = Borer beetle co-occupying the branch shelter made by the ecosystem engineer, ^{D} = Detritivores, ^{G} = Gall inducer that occupies a preexisting gall, ^{H} = Herbivore, ^{I} = Inquiline, ^{L} = Leaf shelter builder co-occupying the shelter made by the ecosystem engineer, ^{N} = Nectar feeder or aphid-tending organism, ^{O} = Omnivore, ^{P} = Predator, ^{S} = Parasitic/Parasitoid. References for: (a) leaf shelter builders: Martinsen et al. (2000), Fukui (2001), Lill and Marquis (2003, 2004), Nakamura and Ohgushi (2003), Crutsinger and Sanders (2005); (b) leaf miners: Hering (1951), Askew (1980), Kahn and Cornell (1989), Kagata and Ohgushi (2004), Szocs et al. (2015), Vieira and Romero (2013); (c) stem borers: Polk and Ueckert (1973), Rogers (1977), Hovore and Penrose (1982), Di Iorio (1994), Paulino-Neto et al. (2006), Calderón-Cortés et al. (2011), Lemes et al. (2015), Gallardo and Cardenas (2016), Novais et al. (2017, 2018); (d) gall inducers: Washburn (1984), Wheeler and Longino (1988), Fernandes et al. (1989), Eliason and Potter (2000), Sanver and Hawkins (2000), Hayward and Stone (2005), Joseph et al. (2011), Almeida et al. (2014), Aranda-Ricket et al. (2017), Santos et al. (2019)

The review of studies reporting the organisms that use plant structures modified by herbivorous engineers (Table 8.1) indicates that secondary users are highly diverse (21 arthropod orders) and comprise herbivores (inquilines, other shelter builder insects, and nectar/honeydew feeders), detritivores/omnivores, predators, and parasitoids. Specifically, the arthropod community associated with leaf shelters includes 19 orders, 116 families, and at least 393 species (Table 8.1); 45% of these families include herbivores (most of them Lepidoptera and Hemiptera insects) and 31% represent natural enemies of herbivores (mainly Arachnida and Hymenoptera). The reported arthropod community associated with branches manipulated by stem borers includes 16 orders, at least 30 families, and ~248 species, of which 46% represent other stem borer species (Cerambycidae, Buprestidae, Bostrichidae) that co-occur in the same branches modified by the ecosystem engineer, whereas the 43% of the species are natural enemies (Arachnida, Hymenoptera, and some coleopteran families) (Table 8.1). Similarly, 44% of the families of secondary users reported for galls comprise Arachnida and Hymenoptera natural enemies (Table 8.1). Previous estimates of secondary users of galls (based on a review of 112 gall systems) indicate a total arthropod richness of 242 species (Sanver and Hawkins 2000). In the case of arthropod communities associated with leaf mines, there are few studies reporting the species of secondary users, which makes it difficult to estimate the species richness, although the available information indicates that 53% of the families associated with leaf mines represent natural enemies (Arachnida and Hymenoptera) (Table 8.1).

Similar to previously reported (Fukui 2001; Sanver and Hawkins 2000; Calderón-Cortés et al. 2011), the data presented in Table 8.1 show that most of the herbivores beneficed by plant modification belong to the same insect order as the ecosystem engineer. Some of these herbivores coexist with the ecosystem engineer (Fukui 2001; Lill and Marquis 2007; Calderón-Cortés et al. 2011), and others use the modified habitat after it has been utilized and abandoned by the ecosystem engineer, particularly those that inhabit vacant leaf shelters and leaf mines (Fukui 2001; Kagata and Ohgushi 2004). Coexisting herbivores (Fig. 8.3), which usually have the same feeding strategy as the ecosystem engineer, use the modified habitat for oviposition and/or feeding. Previous studies have demonstrated that the availability of oviposition sites is one of the main benefits these insects obtain from the ecosystem engineer (Cappuccino and Martin 1994; Lill and Marquis 2004; Marquis and Lill 2007; Calderón-Cortés et al. 2011). The availability of oviposition sites is important to secondary colonizers because the presence of vacant oviposition sites can (i) reduce the costs of searching for suitable oviposition sites; (ii) decrease excavation or shelter building costs; and (iii) reduce exophytic predation during the oviposition period (Fukui 2001; Lill and Marquis 2004; Marquis and Lill 2007; Calderón-Cortés et al. 2011). Nevertheless, one potential negative consequence of using the available oviposition sites in an occupied habitat for colonization is the resulting intra- and interspecific competition (Fig. 8.3; Fukui 2001; Lill and Marquis 2004, 2007; Marquis and Lill 2007: Tack et al. 2009).

Competition among herbivores might be intense because the available food can be limited due to the high densities of occupants or because changes in plant quality

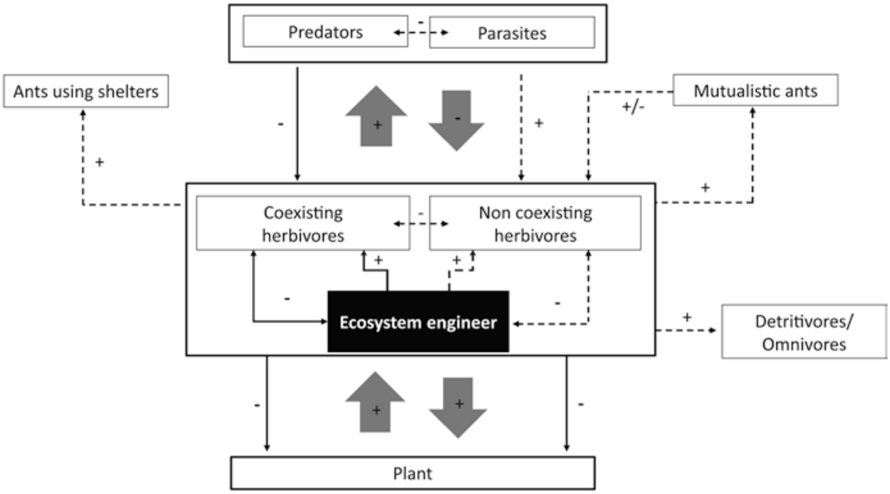

Fig. 8.3 Arthropod community and interactions mediated by herbivorous ecosystem engineers. Positive interactions are indicated by "+", whereas negative interactions are indicated by "–". Continuous lines and arrows represent direct interactions, and discontinuous lines and arrows represent indirect interactions. Organisms belonging to each trophic level are included in the same box. Bottom-up effects are indicated by solid ascendant arrows, and top-down effects by solid descendant arrows

can be detrimental for some species occupying the modified habitat (Lill and Marquis 2007), as has been demonstrated for leaf miners and leaf rollers. Leaf miners compete directly at the leaf level and indirectly through changes in traits of the host plant (i.e., induced response) (Johnson et al. 2002; Tack et al. 2009). Similarly, direct competition related to high densities of conspecifics in the same leaf shelter results in negative effects on insect fitness (i.e., pupal mass and fecundity), which has been demonstrated for some leaf rollers (Lill et al. 2007). Competition effects remain to be examined for most ecosystem engineers, particularly for stem borers.

Sequential colonization of the modified habitats by non-coexisting herbivores is also frequent (Fig. 8.3), particularly in leaf shelters and mines (Fukui 2001). For example, a high number of leaf-chewing insect species have been recorded using abandoned/artificial leaf ties and rolls (Lill and Marquis 2003, 2004; Lill et al. 2007). Some authors propose that most of these herbivores only use leaf constructs as favorable refuges against harsh environmental conditions related to drought (Fukui 2001; Lill and Marquis 2007), and accordingly, it has been demonstrated that some shelters attract more insects during periods of low rainfall (Vieira and Romero 2013; Novais et al. 2018).

Additionally, feces (i.e., frass) and remains (i.e., exuvia, head capsules) can accumulate in the modified habitat as the result of feeding of insect herbivores (Fukui 2001; Lill and Marquis 2004, 2007; Marquis and Lill 2007). Some ecosystem engineers, such as leaf rollers, can occasionally add leaves to the leaf shelter when food becomes scarce (Lill and Marquis 2004). Feces also represent a substrate for microbial colonization (Marquis and Lill 2007). Thus, the accumulation of these resources in the modified habitats increases the availability of food for detritivore and omnivore secondary colonizers. Detritivores/omnivores associated with the habitats modified by the ecosystem engineers (Fig. 8.3) include organisms from Collembola, Diplopoda, Dermaptera, Orthoptera, Psocoptera, and Coleoptera (Table 8.1). These organisms can spend most of their life or stay temporarily in the modified habitat (Fukui 2001), but they are usually free-living secondary users of abandoned shelters/cavities, which implies that they do not directly interact with the ecosystem engineer (Kagata and Ohgushi 2004; Novais et al. 2018). This group of secondary users comprises 12–25% of the organisms associated with the habitat modified by stem borers (Novais et al. 2018), but its abundance is greater in leaf ties built by caterpillars (Lill and Marquis 2004).

Another type of secondary user of habitats modified by some insect engineers is represented by nectar- and honeydew-feeding ants (Table 8.1), which are attracted to galls that secrete sugars (Washburn 1984; Aranda-Rickert et al. 2017), to habitats modified by an aphid, and/or to shelters in which aphids are secondary users (Fukui 2001; Nakamura and Ohgushi 2003; Crutsinger and Sanders 2005; Ohgushi 2008). Leaf shelters on branches with aphid-tending ants (*Formica obscuripes*) had 54% more individuals than shelters on branches without ants, suggesting that aphid-tending ants may actually increase the abundance of arthropods at small spatial scales within leaf rolls (Crutsinger and Sanders 2005). However, negative effects on some insect herbivores (e.g., caterpillars) by aphid-tending ants have also been reported (Nakamura and Ohgushi 2003), indicating simultaneous positive and negative effects on different members of the arthropod community (Fig. 8.3).

The increase in the abundance and species richness of herbivores and detritivores in the modified habitats can be explained by an increase in oviposition sites and refuges and better quality food resources promoted by ecosystem engineering, also known as “bottom-up effects” (Fig. 8.3; Martinsen et al. 2000; Bailey and Whitham 2003; Ohgushi 2005, 2008; Utsumi et al. 2009; Uesugi et al. 2016). However, given that herbivores and detritivores represent potential preys and hosts for their natural enemies (i.e., predators and parasitoids), bottom-up effects propagate upwards to the third trophic level. Interestingly, natural enemies are among the organisms most beneficed by ecosystem engineering, since they represent a great proportion of the arthropod communities associated with the modified habitats (Table 8.1). A quantitative evaluation of the effects of ecosystem engineering on predators (Cornelissen et al. 2016) indicates an increase of 165% and 120% in species richness and abundance, respectively.

Once established, natural enemies can exert top-down effects and control the abundance of herbivorous and detritivorous species (Fig. 8.3) (Paine 1966; Ripple et al. 2016) both interacting directly with the modified habitat (for references see Table 8.1) and in the areas next to the modified habitat (Wetzel et al. 2016). This indicates that ecosystem engineering by insect herbivores plays a key role in mediating trophic cascades and structuring the communities associated with the modified habitats (Martinsen et al. 2000; Fukui 2001; Bailey and Whitham 2003; Lill and Marquis 2003, 2004; Calderón-Cortés et al. 2011; Cornelissen et al. 2016).

Most insect engineers can also have important impacts on ecosystem processes, such as nutrient cycling, the alteration of tree architecture, vegetation composition and hydrology in forests, and ecosystem resilience (Jones et al. 1994; Feller and Mathis 1997; Bailey and Whitham 2003; Martínez et al. 2009; Utsumi and Ohgushi 2009; Marquis 2010; Calderón-Cortés et al. 2011, 2016). Given that the ecological and ecosystem impacts are higher than those expected on the basis of the ecosystem engineer biomass, ecosystem engineering by most insect herbivores such as gall inducers (Bailey and Whitham 2003), leaf rollers (Lill and Marquis 2003, 2004), and stem borers (Calderón-Cortés et al. 2011, 2016) is considered a keystone process (sensu Power et al. 1996).

Overall, the evidence reviewed here confirms that ecosystem engineering by insect herbivores enhances arthropod diversity in terrestrial ecosystems. The provision of diverse resources and higher-quality food (Marquis and Lill 2007; Calderón-Cortés et al. 2011), the increase in habitat heterogeneity (Fukui 2001; Ohgushi 2005; Lill and Marquis 2007; Cornelissen et al. 2016), the establishment of a complex web of negative and positive interactions (Price et al. 1986; Ohgushi 2005; Zhong et al. 2017), and the extended indirect effects to organisms that do not directly interact with the engineer (Novais et al. 2017, 2018) might explain the profound impacts that herbivorous engineers have on biodiversity.

Evolutionary Consequences of Ecosystem Engineering

In addition to its importance on an ecological scale, ecosystem engineering is a significant evolutionary process because it may elicit or modify selective pressures acting on the ecosystem engineer and/or the organisms that use the modified habitat in subsequent generations (i.e., ecological inheritance) by determining which alleles or genotypes have the highest fitness (Odling-Smee et al. 1996; Laland et al. 2017). In this scenario, ecosystem engineering alleles might drive themselves to fixation, leading to a stabilizing selective pressure on the traits related to the ability to modify the habitat in the ecosystem engineer and on the traits that allow organisms to use the modified habitat in other organisms (Laland and Boogert 2010). Currently, there are no empirical studies demonstrating these theoretical predictions, although several lines of evidence suggest that ecosystem engineering might have important evolutionary consequences. For example, it has been reported that Synergini wasp inquilines of oak cynipid gall inducers have lost the ability to induce their own galls as a consequence of specialization in the use of preexisting galls, even though they retain the ability to induce the development of larval chambers lined with nutritive cells (Hayward and Stone 2005). In addition, almost all parasitoids reared from cynipid galls are specific to their hosts, but it is assumed that parasitoid communities initially consist of polyphagous parasitoids that become increasingly specialized over time (Askew and Shaw 1986; Hayward and Stone 2005).

Furthermore, depending on the degree of specialization in the use of the modified habitat and on the interaction strength among the species, it is possible that some interactions promoted by ecosystem engineering are involved in ongoing coevolutionary processes (Laland et al. 2016, 2017). Coevolution or "sequential radiation" among host plants (Solidago: Asteraceae), gall inducers (tephritid flies and moths), and natural enemies (Coleoptera: Mordellidae) has been reported (Nason et al. 2002; Abrahamson et al. 2003; Blair et al. 2005). Because natural enemies, particularly parasitoids, associated with the ecosystem engineers of different guilds belong to the same families (Table 8.1), it is possible that these evolutionary processes might be common among the interaction webs mediated by herbivorous engineers, but this needs to be addressed in future studies.

Factors Affecting Ecosystem Engineering by Insect Herbivores

Despite the great number of studies analyzing factors that determine the abundance and distribution of insect herbivores, little is known about the plant factors that influence the level of habitat modification carried out by herbivorous ecosystem engineers (Marquis and Lill 2007; Cornelissen et al. 2016). A small number of studies have shown that the host plant genotype determines the abundance of galls (Martinsen et al. 2000; Bailey and Whitham 2003; Crawford et al. 2007). Host plant genotypic variation also determined the inquiline diversity of midge-induced leaf galls at local and regional spatial scales; specifically, gall quality and gall size account for ~25–30% of the herbivore diversity (Crutsinger et al. 2009). In addition, plant quality traits such as nitrogen content and the protein-binding capacity of leaf extracts (Marquis and Lill 2010) as well as plant architectural traits such as the number of touching leaves (Marquis et al. 2002) were strongly related to the percentage of colonization of host plants by leaf-tying caterpillars. A high content of nitrogen in female branches has also been suggested as a factor explaining the preferences regarding branch removal by a borer beetle acting as an ecosystem engineer (Uribe-Mú and Quesada 2006). This highlights that plant quality traits can be important factors that influence the abundance and distribution patterns of herbivorous ecosystem engineers. However, further studies are needed to identify other plant traits and factors affecting most ecosystem engineers and arthropods using modified habitats.

Concluding Remarks and Future Directions

Over the last two decades, an important number of experimental studies have demonstrated that ecosystem engineering by insect herbivores has strong positive effects on biodiversity and mediates a complex web of interactions through both bottom-up and top-down effects. As reviewed here, these bottom-up and top-down effects result in a highly structured arthropod community that includes a great diversity of organisms representing similar trophic groups (coexisting herbivores, non-coexisting herbivores, detritivores, mutualistic and shelter-using ants, and natural enemies) and similar species richness among the four different guilds of ecosystem engineers, indicating common patterns of community organization and predictability of the effects that this process has on biodiversity.

Although ecosystem engineering plays a key role in structuring arthropod communities in terrestrial ecosystems, much information about particular ecological interactions is needed. For example, there are no studies evaluating competitive interactions (direct and indirect) among herbivores that have been established in most engineered habitats. Additionally, studies evaluating mutualisms and facilitation of ecosystem engineering in ants and detritivores are scarce, especially those that analyze the quality and biomass of resources available in the modified habitat. The study of these interactions is important to determine which resources have a major influence on the community structure and to identify the plant traits that promote indirect interactions in the context of multispecific interactions. It is also important to conduct studies evaluating the effects and specificity of natural enemies on the arthropods associated with the modified habitats to understand which and to what extent natural enemies mediate the diversity patterns observed and the evolution of the highly structured communities associated with ecosystem engineering by insect herbivores. In addition, it is necessary to determine the effects of the ecosystem engineering process on soil microorganism diversity and the contributions of such microorganisms to nutrient cycling and ecosystem resilience.

Similar to the interactions promoted by other insect herbivores, interactions promoted by ecosystem engineering can vary at different spatial and temporal scales. However, there are no studies evaluating these patterns of variation. As has been previously recommended for endophytic engineers with low dispersal abilities, it is necessary to incorporate metacommunity models to understand the spatial patterns of species diversity and trophic dynamics mediated by ecosystem engineering (Crutsinger et al. 2009). In this sense, it is also important to identify the biogeographical patterns of insects acting as ecosystem engineers. Niche modeling represents an important field of research that can provide valuable information about factors determining the distribution of ecosystem engineers.

Based on the large number of species estimated to exist in each guild of herbivore engineers, it is also important to study other species acting as ecosystem engineers, especially those belonging to feeding guilds that have been poorly studied, such as leaf miners and stem borers. Stem borer and leaf miner endophytic insects represent feeding guilds that differ in their ability to improve the nutritional quality of the tissue of their host through selective feeding and physical manipulation, strategies that might be considered in a continuum of the ability to manipulate host plant quality. Thus, their inclusion in comparative studies of plant manipulation mechanisms will allow to understand the evolution of ecosystem engineering in insect herbivores and to test specific hypotheses about the adaptive significance of the endophytic lifestyle. Indeed, a recent study looking for commonalities among endophytic insects (gall inducers and leaf miners that induce the production of green islands) found several similarities among the plant manipulation mechanisms exhibited by these insects such as the alteration of source-sink relationships, control of amino acid and sugar composition, alteration of cell walls at feeding sites, and changes in phytohormone profiles (Giron et al. 2016). It is possible that borer beetles share some of these mechanisms (Calderón-Cortés et al. 2016), but there are no studies evaluating this topic.

The identification of the mechanisms and molecules involved in plant manipulation is crucial to understanding the evolution of the ability to modify or create habitats, to identify traits associated with ecosystem engineering, and to study their genetic variation among populations. Linking genetic variation in ecosystem engineer traits and fitness consequences with the strength of the interactions promoted in local patches is a promising area of research for the study of the evolutionary consequences of ecosystem engineering, particularly for the study of the geographic variation in coevolution among the ecosystem engineers and some of the organisms that depend on this process. Plants modified by herbivorous ecosystem engineers also represent excellent study models to test several hypotheses pertaining to community genetics (i.e., the effect of genetic variation in plant traits on the distribution of richness and abundance of associated community members) (Whitham et al. 2003). Undoubtedly, the recent advances in functional and ecological genomics will contribute significantly to these research areas, but the construction of a theoretical framework related to ecosystem engineering by insect herbivores will remain as an important challenge for specialists of diverse fields in the decades to come.

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Chapter 9
Sex-Biased Herbivory and Its Effects on Tritrophic Interactions

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Abstract The dioecious plants are organisms whose sexual organs divided into different individuals of the same species, leading to as a response a difference in the morphology, physiology, and genetics of plants. Although some doubts remain about how the sex of individuals is determined, it has been observed that intraspecific variation, mainly the allocation of nutrients and plant defense, influences the selection of herbivores and higher trophic levels. In the case of herbivory, a preference for male plants has been reported, although the performance and survival of herbivores is not affected. However, recent studies have shown that female plants are also subject to strong herbivory. The most recent investigations evaluating dioecism in plants and their effect at higher trophic levels; in spite of the results with Coleoptera, aphids, and Lepidoptera and different predators (omnivore, parasitoid, and natural enemies), it is not known if it is the sex of the plants or the abundance of herbivores that mediates the interaction. More studies are needed with tropical systems in order to evaluate the role of plant sex on herbivore performance and its effect on the third tropic level and the insect community associated as well. The formation of separate sexes in different individuals has been observed in all animal world. Dioecy in plants is still under investigation in evolutionary and ecological terms because it has several missing data about its origin and maintenance. To what extent intraspecific variation affects the plant and higher trophic levels, and what would be the effect of sex on other guilds? Is it possible to correlate sex and network of interactions that surround it? We hope that these questions, among others, will generate curiosity to know the effect of dioecism.

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> Dioecious plants, however fertilised, have a great advantage over other plants in their cross-fertilisation being assured. But this advantage is gained…with some risk…of their fertilisation occasionally failing. Half the individuals, moreover, namely, the males, produce no seed, and this might possibly be a disadvantage… dioecious plants cannot spread so easily as monoecious and hermaphrodite species, for a single individual, which happened to reach some new site, could not propagate its kind… Monoecious plants also can hardly fail to be to a large extent dioecious in function, owing to the lightness of their pollen and to the wind blowing laterally, with the great additional advantage of occasionally or often producing some self-fertilised seeds. When they are also dichogamous, they are necessarily dioecious in function. Lastly, hermaphrodite plants can generally produce at least some self-fertilised seeds…When their structure absolutely prevents self-fertilisation, they are in the same relative position to one another as monoecious and dioecious plants [except] that every flower is capable of yielding seeds. (Darwin 1876)

Plant Dioecy

Sexual reproduction in plants includes a vast array of reproductive structure variations, from hermaphroditism (bisexual flowers) to dioecious plants, which includes indeed plants with male and female flowers in the same individual (monoecious), with hermaphrodite flowers and male flowers (andromonoecious), and with hermaphrodite and female flowers (gynomonoecious) (Avila-Sakar and Romanow 2012; Barrett and Hough 2013; Harkess et al. 2015). Dioecious plants show a physical separation in the sexual primary traits, which separates the two sexes in different plants (Cepeda-Cornejo and Dirzo 2010; Barrett and Hough 2013; Xiong et al. 2016).

Between 5% and 6% of all the plant species on Earth are dioecious, with 15,600 species in 987 genera including 175 different families (Renner 2014). Dioecy has been registered in liverworts (75%), leafy mosses (50%), and gymnosperms (36%), but in angiosperms it is rather rare (6%) (Ming et al. 2011). In this regard, angiosperms are the most studied organisms in this topic so far; this is because of their easy identification and sex differentiation in the field (Renner 2014). Despite these is, the amount of species that are discovered every day change regularly, most of the time because new studies showing that species that were registered or identified as dioecious are not. Examples of these changes are the subdioecius species or cryptic dioecious species, which are plants that maintain the two sexual structures, but one of them is not functional; the cacti *Opuntia robusta*, *Mammillaria dioica*, and *M. blossfeldiana* are examples of them (Parfitt 1985; Strittmatter et al. 2002; Avila-Sakar and Romanov 2012; Mandujano et al. 2014).

In particular, the genera with numerous dioecious species are *Salix*, *Pandanus*, *Diospyros*, and *Litsea* (Avila-Sakar and Romanov 2012). Nevertheless, only 1% of those plant species have been studied (approximately 250 different species) and are mostly in temperate zones (Figs. 9.1 and 9.2).

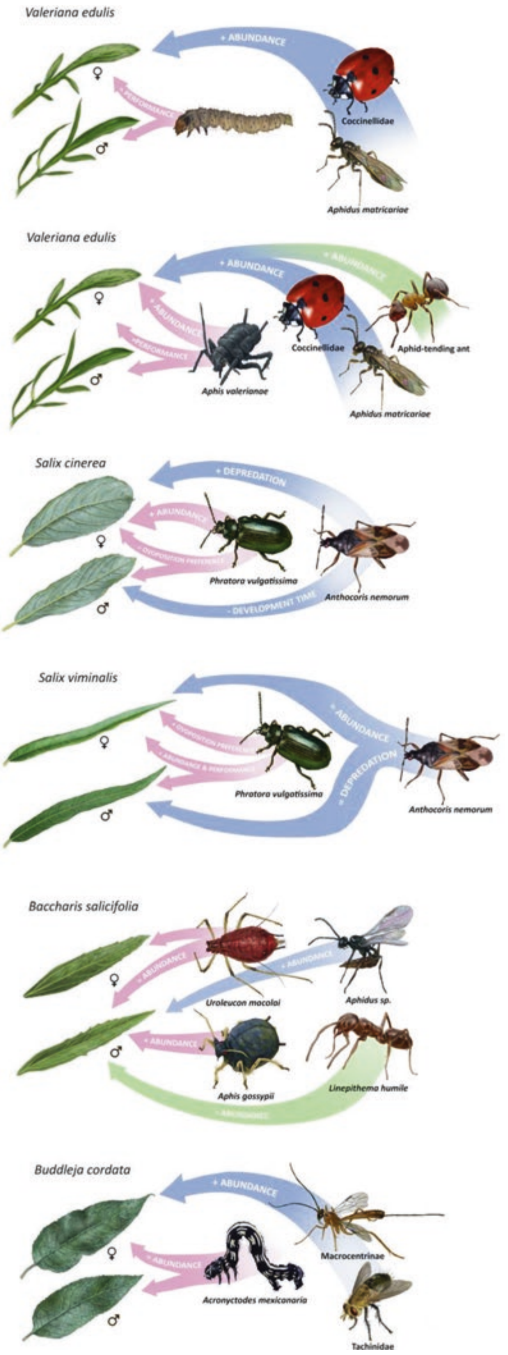

Fig. 9.1 Different traits of dioecious plants result in effects of plant sex on the abundance, damage, and performance of herbivores in relation to herbivores' feeding guild. According to Ågren et al. (1999) and Cornelissen and Stiling (2005) herbivory and abundance by folivores and gall makers is expected to be higher in male plants

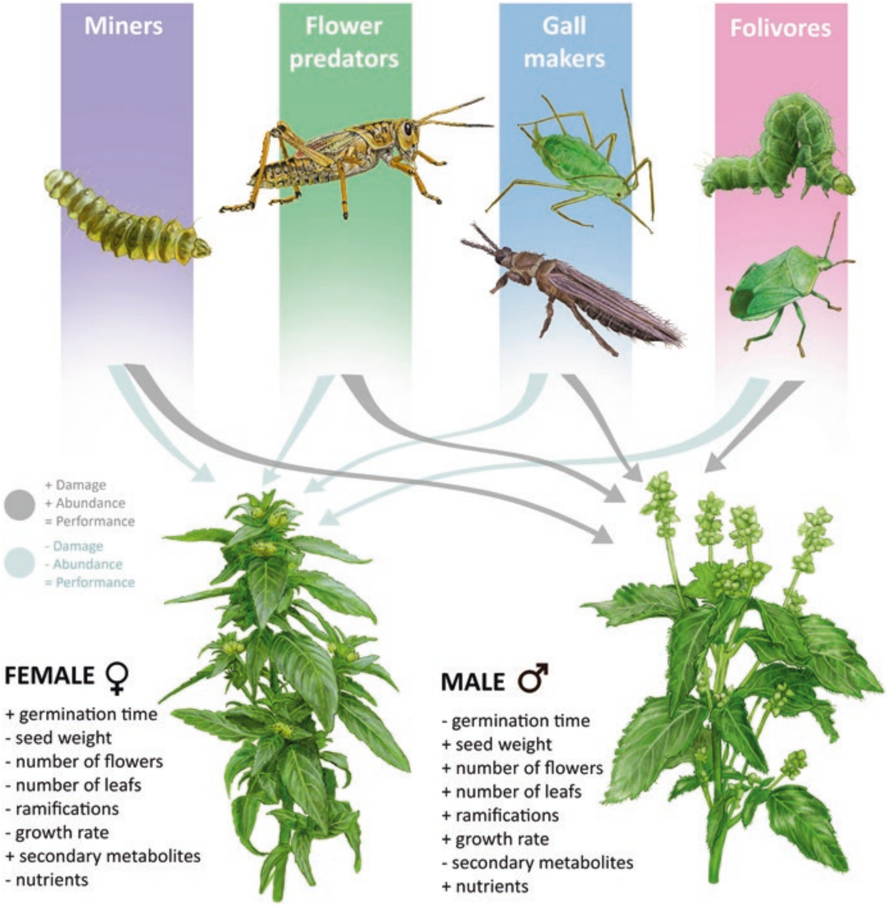

Fig. 9.2 Sex-biased plant-herbivore and predator-herbivore interactions. An increase in the abundance of herbivores in female plants is observed; however developmental time and abundance of predators differ in each case

The study of the effects of dioecy from an ecological and agronomical point of view has increased during the last 20 years (Cornelissen and Stiling 2005). However, evolutionary advantages of this reproduction mode are not well represented in the literature yet, what are the effects of dioecy on the community related to both sexes? whether some species prefer to feed on one plant sex over the other? And how plant defense is allocated depending on the plant sex. In the next sections, we will discuss about the studies that examine the effects of dioecy on the ecological scenario, more specifically on the relationship between dioecy, the herbivores, and the third trophic level.

Evolution of Dioecy in Angiosperms

In 1876, *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom* was published by Charles Darwin, and although the main objective of the book was the sexual reproduction in plants, Darwin already described the complexity of the crossing system in dioecious reproduction. He discussed about the success of outcrossing; however, he raised a decrease in the reproductive success in male plants and a limitation in the plant propagation due to this reproduction mode.

Nowadays, we have evidence of the appearance of dioecious reproduction 871 times independently in the course of the evolution of angiosperms (Renner 2014), which is considered the most important transition in the evolution of reproductive systems (Barret et al. 2010).

There are two hypotheses to explain the evolutionary origin of dioecy. The first one is related to gene mutations. Charlesworth and Charlesworth (1978) suggest that at least two mutations are necessary. The first mutation one is the male function sterilization, giving as a result a population with female plants and hermaphrodite plants. The second mutation suppress the formation of female reproductive structures, giving as a result an hermaphrodite+ male + female population (Charlesworth 2013; Golenberg and West 2013; Käfer et al. 2014; Renner 2014).

The second hypothesis involves an evolutionary pathway from monoecy, gynodioecy, or heterostyly, and it was considered for a long time as the evolutionary origin of dioecious plants. In this case, the proportion of female and male flowers in each individual of a monoecious population is gradually altered, until finally there are only individuals with unisexual flowers (Ainsworth 2000; Barrett 2002). The *Cotula* genus is an example of this mechanism. Another variant is based on a heterostyly pathway. This is particularly observed when the distylous taxa are self-incompatible. The male flowers are derived from short-styled floral morph and female flowers from long-styled morph, as it seems to have happened in some genera of Rubiaceae (e.g., *Cordia*, *Coussarea*, *Psychotria*, *Mussaenda*, and *Erythroxylum*) (Bawa 1980 and references cited therein).

Regardless of the evolutionary pathway, there is a consensus that unisexual flowers evolved from hermaphrodite ones (Charlesworth 2013; Golenberg and West 2013; Renner 2014), and some generalities in angiosperm clades rich in dioecy species are notorious. For example, it has evolved in clades with low floral displays; wind-water and unspecialized insect pollination; small flowers; fleshy fruits; and frugivore-dispersed seeds (see Renner and Ricklefs 1995, and Bawa 1994 for tropical species). Later, Vamosi et al. (2003) carried out a phylogenetic analysis and corroborated a strong correlation between dioecy and abiotic pollination, inconspicuous flowers, and fleshy fruits and also with tropical distribution and woody growth form.

Heilbuth (2000) considers dioecious taxa as evolutionary dead-end points, because they are often at the tips of phylogenetic trees and their richness is lower than that of their hermaphroditic sister groups. Regardless of the tendency to have fewer species, dioecy increases diversification rates, albeit moderately, in clades that contain this reproductive system (Käfer et al. 2014). Nevertheless, for *Allocasuarina* (Casuarinaceae), *Dodonea* (Sapindaceae), *Fragaria* (Rosaceae), *Fraxinus* (Oleaceae), *Gallium* (Rubiaceae), *Lepechinia* (Lamiaceae), *Momordica* (Cucurbitaceae), *Rhus* (Anacardiaceae), and *Sidalcea* (Malvaceae), dioecy has a stronger effect on the diversification rate, whereas for *Gunnera* (Gunneraceae) and *Pilea* (Urticaceae), the effect is negative (Sabath et al. 2016).

There is evidence for and against that diversification rates are increased in groups with dioecious species. However, it is important to note that the positive effect of dioecy on diversification rates could be characteristic of angiosperms, since studies conducted in hornworts (Villarreal and Renner 2013), conifers (Leslie et al. 2013), and mosses (McDaniel et al. 2013) do not show an increase in diversification rates as a consequence of dioecy, although these groups have a higher percentage of dioecious species.

Evolution of dioecy has been analyzed mainly from the point of view of the floral reproductive systems per se; however it is known that the plants are subject to daily herbivory, which imposes a strong selection pressure (Lucas-Barbosa et al. 2011; Schiestl et al. 2014). Preliminary observations made by Ashman (2002) pointed out for the first time the importance of herbivory as an evolutionary force that may lead to dioecism. Their research with *Fragaria virginiana* showed that hermaphrodite flowers were more attacked by herbivores than female flowers and the risk of herbivore damage increases toward male function.

Complementary to the proposal of Ashman (2002), there are some studies that show that the evolution of dioecy can be mediated by sex-biased herbivory, as a consequence of the inherent difference of the allocation of resources to the male and female floral morphs (reviewed in Avila-Sakar and Romanow, 2012). Although for Avila-Sakar and Romanow (2012) male-biased herbivory should not be taken as a rule. They recommend expanding the study of dioecy following a standardized protocol with appropriate tests for sexual dimorphism of (a) the cost of reproduction, (b) vegetative growth, and (c) allocation to defense, as well as accounting for other factors, such as habitat.

How Different Are They? Outside and Inside Dioecious Plants

Dioecious plants historically have provided excellent examples to examine tradeoffs in resource allocation related to plant reproduction (Cepeda-Cornejo and Dirzo 2010). In these species, reproductive allocation is frequently asymmetrical between individuals of the two sexes, which promotes the formation of different plant traits that plants develop according to their three main vital functions for life: reproduction, growth, and maintenance (e.g., defense) (Obeso 2002; Barrett and Hough 2013).

In terms of reproductive costs, most of the sources are allocated to flower production, for example, it is well known that female plants require a higher amount of nutrients during the fruit and seed production than their male counterparts (Antos and Allen 1999; Suzuki 2005). On the other side, male plants invest a higher amount of nutrients to produce a higher aerial biomass, higher amounts of nectar per flower, and a higher pollen production and for the traits responsible to attract pollinators (Obeso 2002; Ueno et al. 2007; Barrett and Hough 2010; Renner 2014). Actually, if we consider the entire reproductive season, females invest more sources during the entire season because their allocation on the formation and maturation of the fruits and seeds is higher (Cepeda-Cornejo and Dirzo 2010). In counterpart, male plants allocate more sources for the growth and development of vegetative tissues, for example, the seed weight and size of spinach ((*Spinacia oleraceae* (Chenopodiaceae)) and from the snow dock (*Rumex nivalis* (Polygonaceae)) are higher in male plants compared to female plants of both species.

In other plant traits, males also outperform females in growth rates, nutrient contents, and the leaf sizes (Barrett and Hough 2013; Renner 2014; Wilson 2016). For example, the Spanish juniper (*Juniperus thurifera* (Cupressaceae)) showed no differences on the growth rings during the first stages of their development between sexes; nevertheless, when they reached the reproductive stage, female plants exhibited a reduction in their growth (Montesinos et al. 2006; Torres 2007). Other cases are the female plants of *Siparuna* (*Siparuna grandiflora* (Siparunaceae)) and the common holly (*Ilex aquifolium* (Aquifoliaceae)) female plants of both species had a faster growth rate in a pre-reproductive stage compared with male plants (Obeso 2002).

During the reproductive stage, several plant traits such as the inflorescences number, size and number of flowers, longevity, nutrients content, and anti-herbivore defenses can show very pronounced differences between male and female plants (Barrett and Hough 2013). From the physiological point of view, the chemical production and the secondary metabolites composition differ between tissues in dioecious species showing again an asymmetrical resource investment (Ågren et al. 1999; Avila-Sakar and Romanow 2012; Barrett and Hough 2013).

Nutrients such as nitrogen, phosphorous, carbon, calcium, and magnesium tend to be present in higher amounts in female plants, while male plants have shown a better water absorption (Fernandes 2016). Regarding secondary metabolites, it is hypothesized that plants with faster growth rates (male plants) allocate less resources in physical and chemical defense. Because female plants have slower growth rates, they produce a higher amounts of defense metabolites as a response against the loss of foliar tissue through herbivory (Cornelissen and Stiling 2005; Cepeda-Cornejo and Dirzo 2010; Avila-Sakar and Romanow 2012; Espírito-Santo et al. 2012). Overall, male plants are more vigorous and produce a higher amount of leaves, their vegetative growth is faster, and they show a low investment in chemical/physical defenses; on the other side, female plants are smaller, and they produce fewer leaves and show higher accumulation of nutrients that can be used for defense or reproduction (Obeso 2002; Cornelissen and Stiling 2005; Cepeda-Cornejo and Dirzo 2010; Avila-Sakar and Romanow 2012).

Who Is Eating Whom? The Gradient of Responses Depends on Herbivore Identity But also on the Plant Structure on Which Herbivores Feed

As we mentioned before, dioecious plant can be an important source of genotypic variation (Ågren et al. 1999; Cornelissen and Stiling 2005; Mooney et al. 2012; Abdala-Roberts et al. 2016; Moreira et al. 2019), which is given mainly by two factors: environment and the genotypes of the plants. It has been demonstrated that the plant intraspecific genetic variation can affect the structure of associated arthropod communities (Hare 2002; Hughes et al. 2008; Mooney and Singer 2001). The different genotypes of a certain plant species can host herbivore communities that can be significantly different between sexes, for example, in the density, uniformity, and species diversity, and in the case of the interactions, this is not the exception (Mooney et al. 2012; Abdala-Roberts et al. 2016; Nell et al. 2018).

What is well known so far is the effect of physical and chemical plant traits and their negative effects against insect herbivory. A hypothesis constructed with this evidence has received the name of the "sex-biased herbivory hypothesis" (Ågren et al. 1999; Cornelissen and Stiling 2005; Granados-Sánchez et al. 2008; Kabir et al. 2014). This hypothesis recognizes variation in the quality of the plant, where male plants are mostly attacked by insect herbivores due to their increased energy expenditure in growth (biomass), in contrast to the female plants whose investment is allocated to the reproduction and the production of organic defensive compounds (Ribeiro-Mendes et al. 2012; Cornelissen and Stiling 2005). Previous reviews conducted by Boecklen and Hoffman (1993) and Ågren and collaborators (1999) showed a tendency of a biased herbivory toward male plants. Among the most important results that Ågren and collaborators (1999) reported were a greater herbivory in leaves, bark, buds, and flowers of male plants in 21 dioecious species from 11 different families.

Cornelissen and Stiling (2005) in a more quantitative way evaluated the effect of the sex-biased herbivory in different guilds (33% for gall-forming insects, 31.3% for folivorous insects, and 34% for others (flower predators, pathogens, leaf miners) by conducting a meta-analysis. Their results indicate that the herbivory inflicted by folivores and gall makers is higher in male plants, with the exception of pathogens, whose tendency is toward female plants. In addition to this, their analysis showed that there is greater herbivore abundance in leaves and stems of male plants.

Although male-biased herbivory seems to be the most recurrent pattern reported so far, some authors consider that this is not a generalization because most of the studies reported a tendency for only few plant families, mainly the genus *Salix* (1) because these plants have an economic importance and (2) because they are easy to differentiate between male and female plants in the field. Coupled with this, another issue that has not been explored deeply is the early ontogenetic stages between plant sexes (Cornelissen and Stiling 2005; Ueno et al. 2007; Ávila-Sakar and Romanow 2012). Studies conducted on in *Lomandra longifolia* (Lomandraceae) (Ahmad et al. 2008) and in Arecaceae (Castaño et al. 2014, 2016) showed that in these dioecious species, flowers are bisexual in the first stages of organ formation and subsequently the mature flowers became functionally unisexual due to the abortion of male/female organs. Recently, it has been demonstrated that the floral development of male and female flowers can be mediated by DNA methylation, like in the dioecious *Fraxinus mandshurica* (Oleaceae) and *Salix viminalis* (Salicaceae) (Zhu et al. 2016; Cheng et al. 2019).

Another constraint comes from the number of investigations that have been reported, Ågren and collaborators (1999) cautioned about publication bias which may occur because of a tendency by reviewers and editors to reject studies that fail to find significant differences between sexes in their responses to herbivory. In addition to other recently published research in which female-biased herbivory was found, for example, Maldonado-López et al. (2014) found higher levels of herbivory in female plants, which was attributed to differences in nutritional content in the Mexican ciruela (*Spondias purpurea* (Anacardiaceae)). In *Spinacia oleracea* (Chenopodiaceae), it has been reported that herbivory damage is greater in female plants, but only when they are competing among plants of the same sex (Pérez-Llorca and Sánchez 2019). Additionally, the female plants of *Ilex glabra* (Aquifoliaceae) present greater leaf damage, but only after flowering (Buckley and Avila-Sakar 2013). Finally, African elephants prefer to feed on the female marula trees (*Sclerocarya birrea* ssp. *caffra* Anacardiaceae) because the branches are shorter and less ramified than in male trees (Hemborg and Bond 2007). Then an early conclusion can not be drawn due to the variability between the systems, so more research by using dioecious species is urgently needed.

Another issue that is covered in the “sex-biased herbivory hypothesis” is from the attacker perspective. Herbivore performance and survival show significant differences depending on the sex of the plant on which they feed; this is a result of the differential amount of nutrients allocated between sexes. It is expected that male plants provide a greater amount of nutrients, because they are more vigorous, with large leaves and greater vegetative growth, being large contributors of nutrients that allow a higher and faster development of herbivores (Cepeda-Cornejo and Dirzo 2010).

In the case of plant defense, it can be direct through the production of toxic chemical substances such as alkaloids, phenolic compounds, quinones, etc. whose effect is to kill or elongate the developmental time of the herbivores (War et al. 2012; Fürstenberg-Hägg et al. 2013). For instance, those plants that release lower concentrations of secondary metabolites will promote a greater herbivore survival and performance.

Particularly, for dioecious plants a preference by herbivores and a higher foliar damage were detected to be biased to male plants (Cornelissen and Stiling 2005). Cuda et al. (2018) confirmed this assertion; they found that the average mortality time in the weevil *Apocnemidophorus pipitzi* (Coleoptera: Curculionidae) feeding in female plants of the Brazilian peppertree (*Schinus terebinthifolius* (Anacardiaceae)) is lower, in comparison with the mortality time in weevils feeding on male plants. The same authors documented that female plants from this peppertree species have a stronger aroma and lower levels of herbivory compared to male plants. The meta-analysis performed by Cornelissen and Stiling (2005) showed no significant differences in the herbivore survivorship between the two sexes. So far, the reviews seem to show an herbivore preference toward male plants; however it is not possible yet to draw a general conclusion. Controlled performance experiments with the herbivores seem to be the way to evaluate this; nowadays rearing of insects is a very affordable technique, and this could help researchers to understand the insect performance, survival, and preference in the dioecious plants.

Plant Sex as a Mediator of Interactions. What Is Going on at the Tritrophic Interactions Mediated by the Sex-Biased Herbivory?

Despite the extensive study on herbivory in dioecious species, there is very little empirical evidence on its effects on the third trophic level (natural enemies of herbivores: parasitoids and/or predators) (Mooney et al. 2012; Moritz et al. 2017). Recent data suggest that the variation in the nutritional quality of vegetative tissues, the availability of floral and extrafloral nectar, structural defenses, and communication by the release of volatile organic compounds (VOCs) or herbivore-induced plant volatiles (HIPVs) are communication mechanisms toward higher trophic levels, and all of them have been classified as indirect defenses in plants (War et al. 2012; Fürstenberg-Hägg et al. 2013; Aartsma et al. 2017). After the discovery of indirect defenses, an explanation emerged; it mentions that plants function as mediators on the abundance and richness of herbivores associated to plants. A bottom-up effect in where the indirect interactions between plants and natural enemies of the herbivores affect negatively not only the dynamic of interactions between herbivore-predator but also the community composition associated to the plant. This explanation was described as plant trait mediated by Oghushi and collaborator (Utsumi et al. 2010a; Ohgushi et al. 2012).

Investigations that evaluate the plant trait mediated based on evaluating specific interactions consist of three main components: an initiator, a mediator, and a receiver. Thus, the consumption of insect herbivores (initiator) will induce changes in plant characteristics and a differential response in male and female pants (mediator), affecting the performance of the plants and promoting the release of the defensive compounds, which in turn has an impact either negatively or positively in growth and survival abundance of the herbivores and their predators (receiver) (Utsumi et al. 2010b).

Although research developed on plant trait-mediated interactions is well represented in the literature, very few studies examine this relationship by using dioecious plants. Most of the studies so far compare differences in abundance, developmental time, and survival of both the herbivores, predators, and parasitoids feeding on plants of both sexes (Mooney et al. 2012; Petry et al. 2013, Kabir et al. 2014; Abdala Roberts et al. 2016; Moritz et al. 2017; Moreira et al. 2019), and only one recent study conducted by Nell and coauthors (2018) evaluate a dioecious plant in terms of its influence on arthropod communities. In this section, we will describe all these studies of tritrophic interactions among dioecious plants, herbivores, and predators (parasitoids) and in the arthropod community context.

Mooney and collaborators (2012) compared male and female Valeriana edulis plants for constitutive and induced direct resistance against two herbivores, the early-season caterpillar *Eanna* spp. (Tortricidae: Lepidoptera) and a late-season aphid *Aphis valerianae* (Hemiptera: Aphididae), and for constitutive and induced indirect resistance in terms of abundance of natural enemies o herbivores and aphid-tending ants. They found no significant differences between sexes in constitutive direct plant resistance. However, they found differences in the indirect resistance, 78% more natural enemies of the herbivores and 117% more ants are found in female compared to male plants. Induction made by early caterpillar herbivory induced direct and indirect resistance in both plant sexes, which in fact increased the developmental time of the caterpillar by 26% and the abundance of the natural enemies by 147%. Interestingly they found no interactions between the two main factors they measured: induction and the plant sex, suggesting that each factor (plant sex and induction) independently influences the performance of the herbivores and the arthropod communities associated to the dioecious plant *V. edulis*.

Petry et al. (2013) by using the same plant model plant focus on the interaction between the aphids, tending ants, and aphid predators in *V. edulis* plants. They conducted a 3-year survey in which they found that female plants are able to maintain 4, 1.5, and 4 times higher densities of aphids, aphid predators, and aphid-tending ants, respectively, compared to males, respectively. Both studies suggest that the factors that influence herbivore abundance and tending ants are (a) the greater attractiveness of female plants via extrafloral nectaries and (b) a density-mediated indirect effect given by the aphid abundance. Finally, the bias for the predators is explained only by the greater attractiveness of female plants.

Kabir and collaborators (2014) evaluated the effect of the dioecious gray willow *Salix cinerea* (Salicaceae), on the interaction between the blue willow beetle *Phratora vulgatissima* (Chrysomelidae) and the carnivorous common flower bug *Anthocoris nemorum* (Anthocoridae). They evaluated abundance, density, and developmental time of the beetle *P. vulgatissima* (herbivore) and density and egg predation caused by *A. nemorum* (predator). The number of eggs laid by the herbivore did not differ significantly between plant sex; however both herbivores an predators, preferred female twigs rather than male plants. Regarding the predator, they found that developmental time was shorter when they were reared in male twigs compared to female twigs. In the field, herbivores preferred female plants, and a density dependent effect was detected by the predators even though herbivores feeding on female plants represent a sub-optimal resource for the common flower bug.

Following the study performed by Kabir and collaborators (2014), Moritz et al. (2017) by using the similar herbivore-predator interaction but with a different plant species, the white willow *Salix viminalis* (Salicaceae), conducted experiments in the laboratory and the field, and what they found was that the herbivore laid more eggs and their predator survival was longer, on female plant, these under laboratory conditions. In the field, however, these results were not the same; for the herbivore, they did not detect differences in the abundances (larvae and adult stages) between both plant sexes, and the same pattern was found for the predator abundance, which was not affected by the sex of the plant.

Abdala-Roberts and coauthors (2016), using the dioecious *Baccharis salicifolia*(Asteraceae) studied above and belowground insect and fungal interactions. They found that the abundances of specialist aphid *Uroleucon macaolai* were not affected by plant sex; however the generalist aphid *Aphis gossypii* and its tending ant (*Linepithema humile*) were more abundant and denser in male plants. In the case of the third trophic level represented by the aphid parasitoids, their abundance was not affected by the plant sex. The same authors also evaluated the arbuscular mycorrhizal fungi, in where they observed that the mycorrhizae abundances were 1.4-fold higher in faster growth plants and the density was higher on plants with slow growth. Female plants had a higher abundance and density of mycorrhizae. Although they did not detect an interaction between the sex of the plant and the growth rate, they suggest a potential influence of the plant sex on the mycorrhizae establishment.

Moreira and coauthors (2019) studied the plant effects on the densities of herbivore *Acronyctodes mexicanaria*(Geometridae) of the dioecious shrub *Buddleja cordata*(Scrophulariaceae) and its parasitoids. They measured several plant traits that could potentially be associated with such effects. They found that plant sex did not affect the abundance of the herbivore; however, they found an effect on parasitoid abundance having 2.4 more parasitoids on female plants. Such an effect of plant sex on parasitoids remained even after including herbivore abundance in the model, suggesting a trait-mediated mechanism driving differences between plant sexes in the abundance of the parasitoids.

Overall, these studies do not show a clear pattern to draw a hypothesis yet. A higher abundance of natural enemies of herbivores (parasitoids or predators) or tending ants has been detected in female plants; however this pattern seems to be driven more by an herbivore density-mediated effect rather than the plant sex itself. Kabir et al. (2014) and Moreira et al. (2015) through their analysis found an interaction between the sex of the plant and the abundance of natural enemies of the herbivores, which suggest that predator attraction depends on the phenotypic variation associated to herbivory; however dioecious plants respond differentially to the foliar damage.

Why damage is higher in female plants? Because these plants accumulate higher amounts of nutrients such as carbon and nitrogen, which are indeed, basic elements in the production and development of VOCs that are used in the attraction of natural enemies of herbivores. On the other hand, defensive compounds liberated after damage are ingested by the herbivores affecting their performance. Godfray (1994) mentioned that the parasitoid's sensorial capacities are highly specific and allow these organisms to detect and recognize odors from their prey when they are feeding. Studies that evaluate the sex effect on the volatile production have not been tested directly yet; however that could be the starting point to elucidate the potential mechanisms that both sexes use in order to maximize the attraction to the natural enemies of the herbivores. Another aspect that we can suggest is the response between the interaction of dioecious plant-herbivores and natural enemies of the herbivores, which seems to be contrary to most of the results found on the bi-trophic context.

What About Community Level?

As we have been describing along this chapter, the separation of reproductive functions in dioecious plants has been hypothesized to drive divergence in source allocation between male and female plants (Lloyd and Webb 1977; Delph 2007), resulting in a sex-biased herbivory. Studies that shows how intraspecific genetic variation is one of the main drivers that affect arthropod communities are well represented in the literature (Hare 2002; Whitham et al. 2006; Bailey et al. 2009; Mooney and Singer 2012; Pratt et al. 2017). Until now, only a couple of studies has explored the effect of sexual dimorphism on plant-associated arthropod communities.

Because of the morphological and physiological differences between sexes in dioecious plants, it is expected that both types of plants interact in different ways with their interactions against herbivores, pollinators, and pathogens. However, when we performed searches using tritrophic interactions in dioecious plants or sex-biased multitrophic interactions, we only detected the next two studies which were published just recently.

Nell and coauthors (2018) by using *Baccharis salicifolia* (Asteraceae) tested genetic variation and sexual dimorphism on plant traits and associated arthropod communities. They found that sexual dimorphism had weaker effects on flower number, relative growth rate for the plant, predator density (50% higher on females), and arthropod community composition; however, effects of plant sex were not detected on herbivore density.

Recently, a study conducted by Tsuji and Fukami (2018) in where they examine the consequences of sexual dimorphism in the dioecious shrub *Eurya emarginata* and *E. japonica* on the nectar microbe community. They found that nectar-colonizing microbes such as bacteria and fungi were more than two-fold as prevalent and more than ten-fold more abundant in male compared to female flowers. In the case of visitation frequency made by animals (including insects), they detected a stronger effect on the animal visitation frequency on microbial communities in male flowers, while the order of arrival affects them more in female flowers.

One question emerges from this scarce evidence: How plant dioecy affects insect communities? If we assume that the phenotypic variation given by this evolutionary process results in morphological and physiological changes in plants from different sex, it is expected then a divergence in the herbivory, infection, and visitation rates that dioecious plants have with the insect community associated and by instance a change in the diversity of the associated organisms. More studies on the effects of dioecious plants on the community of associated organisms to these plants are urgently needed; this in order to elucidate how interactions change depending of the sex of the plant.

Conclusions

Effect on the multitrophic interactions in dioecious plants is still a poorly explored field. Nowadays we have a strong evidence of sex-biased herbivory in a bi-trophic context, being plant traits such as nutrients and direct and indirect defenses as the main drivers in this divergence and which determine the abundance, survival, and richness of the herbivores. However, on the light of the multitrophic interactions, it is not clear yet. The bias occasioned by the study systems, the exclusion of no significant results, and the differentiation between density dependency and sex effects make still difficult to delineate a pattern on this topic. More studies testing the effect of dioecy on the multitrophic context are needed; as we can see, they are very helpful in system such as *Salix* which are important economically species.

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Chapter 10
Functional Plant Traits and Plant-Herbivore Interactions

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Abstract Functional diversity is a key concept to understand how plants respond to selective pressures and how they influence ecosystem processes. Although there is empirical information about the variation of the functional traits of plants in environmental gradients, their use to understand their biotic interactions has been little explored. Currently, novel proposals for the analysis of the plant-herbivore interactions integrating the study of functional traits have been put forward. This approach can help to generate projection models about the effect of herbivory on ecosystem processes under different environmental scenarios. In this chapter we present a review of the concept of functional diversity in plants and their application in herbivory studies, through the use of syndromes and their ecological and evolutionary implications. In addition, we propose some lines of research that can help build a comprehensive framework to relate the functional responses of plants and defense theories, as well as determine the biotic and abiotic factors that regulate herbivory and its impacts on ecosystem processes.

Keywords Functional diversity · Plant traits · Defense syndromes · Herbivory · Ecosystem processes

Different models of social development have led us to a grave environmental crisis in which biodiversity conservation is under serious threat at local, regional and global scales. For this reason, decisive and effective measures are urgently required in order to face the current and future challenges and conserve a considerable fraction of the current biological diversity (Chapin et al. 2000; Dirzo et al. 2014), which

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directly or indirectly provides many benefits for mankind (Díaz et al. 2007). One of the main lines of action is to understand the mechanisms that generate and maintain biodiversity, as well as the manner in which the loss or addition of species can influence ecosystem functioning (Tilman 2000). The classic approach to understanding these processes has been through analysis of the richness and composition of species. However, there is a broad current consensus that ecosystemic processes depend on the type and relative abundance of the functional characteristics of the species, which have evolved through interactions with the biotic and abiotic environment in which their populations have developed (Hooper and Vitousek 1998; Díaz and Cabido 2001; Lavorel and Garnier 2002).

In recent decades, the study of functional diversity has been widely used to elucidate different patterns, such as species distribution, competitive capacities of species, community influence on ecosystem functioning (Orlandi et al. 2015), assemblage rules within the communities (Díaz and Cabido 1997; Cornwell and Ackerly 2009), responses of organisms to extreme conditions (Westoby 1998; Golodets et al. 2009; Orlandi et al. 2015), and biodiversity conservation. However, one little-studied aspect has been the relationship between the functional traits of plants and their biotic interactions, such as herbivory, pollination, and seed dispersion, among others (Díaz et al. 2007; de Bello et al. 2010).

Herbivory is a selective pressure that affects plant adaptation and influences ecosystem processes such as plant succession, nutrient cycling, and the maintenance of floristic diversity (Janzen 1970; Coley and Barone 1996; Schowalter 2000). More than half of the species described to date (plants, herbivores, and natural enemies of the herbivores) are involved in this interaction (Price 1997; Novotny and Basset 2005), and it is therefore considered a key interaction for the plant communities' adaptations (Coley and Barone 1996).

In general, the relationships that exist between the functional traits of plants and their capacity for defense against herbivores have been established, i.e., the capacity of plants for minimizing attack and the negative consequences of consumption of their tissues. The most commonly adopted approaches include those related to individual morphological traits (such as growth form and plant height), nutritional quality (water and nitrogen content), defensive attributes (latex, spines, trichomes, and chemical compounds), phenology, and capacity for regeneration. In most cases, patterns of herbivory in plant communities have been analyzed using one of these approaches in an isolated and discrete manner, i.e., individual strategies are considered under the assumption that energetic trade-offs take place within the plants, leading to investments directed mainly at one of these attributes, with no opportunity to invest in other defensive attributes. Alternatively, another scientific movement recognizes that plants assign resources simultaneously to different defensive characteristics, generating defense syndromes that depend on the balance of the defensive and growth attributes expressed by the plants (Agrawal and Fishbein 2006).

With the predominant vision of analysis of individual plant attributes, it is impossible to identify the functional traits of importance to susceptibility to herbivory in each community and to be able to understand its influence on ecosystem processes (McNaughton et al. 1989; Hartley and Jones 2004; Dubey et al. 2011; Loranger et al. 2012; Metcalfe et al. 2014), as well as its spatial and temporal variation. Integration of functional traits related to plant defense, seen through the perspective of defense syndromes, could be an integral framework with which to relate the theory of plant economic spectrum (i.e., species strategies shaped by their evolutionary history) and the theories of defense and to determine the biotic and abiotic factors that regulate herbivory and its impact on ecosystem processes (Defossez et al. 2018; de Bello et al. 2010; Reich 2014). In this chapter, we conduct a review of functional diversity in plants and its application in the study of plant-herbivore interactions. We summarize some concepts and empirical evidence regarding functional diversity, as well as describing the most novel approaches for a multivariate integration of the functional responses of plants to herbivores. Finally, we suggest some future lines of research.

Functional Plant Diversity: An Overview

The study of functional diversity in plants has its conceptual basis in previous research addressing the classification of vegetation according to different criteria, such as stem height and density of plants, those related to resource use, their life forms, and as a function of environmental stress (Orlandi et al. 2015). This concept as we know it today is the result of a long trajectory that has included different approaches and began to peak the 1990s as a result of the challenges posed by global climatic change (Chapin et al. 2000; deLaplante and Picasso 2011). During this period, there was a strong advance in the standardization of key concepts, such as that of the functional trait, and a broad development of evaluation metrics (Lavorel and Garnier 2002; Cornelissen et al. 2003; Petchey and Gaston 2009; Kleyer et al. 2012).

Currently, the concept of functional diversity is widely accepted and refers to the interval and abundance of functional traits present in a community (Díaz and Cabido 2001). This concept is key to relating the manner in which biodiversity affects ecosystem processes (McGill et al. 2006), through analysis of its functional traits, defined as morphological, physiological, and phenological characteristics measured at an individual level (Díaz and Cabido 2001). Functional traits have been classified into two groups. Firstly, the hard functional traits, which are those that affect the ecosystem processes and directly measure the function of interest (e.g., physiology of the organisms, rates of assimilation, photosynthesis, etc.). Secondly, considering the limitations that exist in terms of the accurate systematic and repeatable evaluation of hard functional traits, the concept of soft functional traits has been adopted, which it is assumed can evaluate the fundamental processes of the organisms in an indirect and easily measured manner compared with hard functional traits (Díaz and Cabido 2001; Hooper et al. 2002; Díaz et al. 2007). For example, leaf hardness is a soft functional trait that can be an indicator of defense, based on carbon and decomposition and mineralization rates. The greater the hardness of the leaves, the greater the defense against herbivory and the lower the rate of decomposition (Poorter et al. 2009; Poorter and Bongers 2006).

There are two general approaches to evaluating functional diversity. The first is discrete and consists of the integration of functional types or groups of species that, without considering their phylogenetic relationships, present similar responses to the environment or similar effects on the ecosystem processes (Díaz and Cabido 2001; Casanoves et al. 2011). The second approach is quantitative and conducted through indices that, in turn, can be classified as a function of the number of traits and the evaluation techniques used (Casanoves et al. 2011; Hooper et al. 2002). The selection of characteristics that suitably represent the processes has not been an easy task; however, it is of vital importance to our understanding of the underlying mechanisms. For this reason, in the last decade, efforts have increased toward the development of a list of characteristics that involve different criteria, including ease of measurement, application to ecosystem processes and understanding of species distribution, as well as information about the cost-benefit relationship and physiology of certain attributes (de Bello et al. 2010; Pérez-Harguindeguy et al. 2003).

At present, it is widely recognized that species richness is not a good indicator of functional diversity. This is due, among other reasons, to the fact that most species are not of equal importance in terms of their contribution to ecosystem processes (Díaz and Cabido 2001). Moreover, it has been recognized that functional composition is more important than functional richness, in terms of understanding the impact of the species on functional processes (Hooper and Vitousek 1998). For this reason, the loss of a functional type could have a greater impact on ecosystem processes that a reduction in the number of species (Díaz and Cabido 2001; Hooper et al. 2005). Among the studies of functional diversity, the most reviewed topics have been the following: (1) the relationship between taxonomic diversity and functional diversity, (2) standardization of concepts and methods of measurement, (3) traits that describe the function of an organism in the ecosystem, (4) factors that determine functional traits (environmental heterogeneity, competition, trophic dynamics, disturbance, etc.), (5) the ensemble of communities, and (6) the level of functionality necessary to maintain ecosystem processes in response to biodiversity loss.

Different studies have identified functional characteristics as those key mechanisms by which species and groups of species influence the properties of the ecosystem (Lavorel and Garnier 2002; Díaz et al. 2004, 2007; Hooper et al. 2005) through biogeochemical processes are related to the cycling of carbon, nutrients, and water, as well as net primary productivity (Díaz et al. 2007; Faucon et al. 2017). This theme is being addressed by studies that identify the functional roles of groups of nitrogen-fixing plants (Spehn et al. 2002), soil engineers (Wardle et al. 2004; Boyero et al. 2007), microorganisms and soil fauna (Bailey et al. 2002; Heemsbergen et al. 2004; Wall 2004; de Bello et al. 2010). While advances have been made in these topics, there is little empirical evidence of the role of functional traits in biotic interactions and their repercussions on ecosystem processes, despite the recognition that these processes are the result of interaction across different trophic levels (de Bello et al. 2010). For example, processes related to soil fertility and carbon capture are affected by the functional composition of plants and soil fauna (Zimmer et al. 2005; de Bello et al. 2010). Pollination is regulated by the interaction of plant and animal attributes (Albrecht et al. 2007; Albor et al. 2019). These studies demonstrate that the functional composition of a trophic level is affected by other associated trophic levels (de Bello et al. 2010).

Linking Herbivory and Plant Functional Traits

Through the approach of functional diversity, it is possible to quantify the responses of organisms to different selective pressures, as well as the effects of these pressures on ecosystem processes. With this information, it is possible to construct projection models of different environmental and climatic change gradients (McGill et al. 2006; Reese et al. 2016). In this context, study of the impacts of herbivory using the functional diversity approach can help to generate models that predict the effects of herbivory on the structure and functioning of the communities of plants under different environmental scenarios (Dubey et al. 2011; de Bello et al. 2010).

Herbivory is a process with important consequences at different levels of organization, such as ecosystems, communities, populations, and individuals (Coley and Barone 1996). For this reason, it is unsurprising that herbivory contributes to different ecosystem processes, prominent among which are the cycling and conservation of nutrients (Price 1987; Singer and Shoenecker 2003; Feeley and Terborgh 2006), succession, vegetal regeneration, recruitment of species (Janzen 1970), plant survival, growth and reproduction (Crawley 1983; Dirzo 1984), and therefore community structuring (Janzen 1970; Connell and Slater 1977; Crawley 1983; Dirzo and Miranda 1990).

Due to the negative impact of herbivores on plants, it has been suggested that herbivory has the potential to function as a selective pressure that generates adaptive responses on the part of the plants (Marquis and Braker 1994; Price 1997; Crawley 1997), including chemical and physical defenses and mechanisms of attraction of the natural enemies of herbivores (e.g., parasitoids, extrafloral nectaries) (Agrawal and Konno 2009, Ness et al. 2009). Plant responses to herbivory can be categorized into two general strategies: resistance (the capacity to minimize the loss of tissue to the herbivores) and tolerance (the capacity to recover lost tissue). The capacity of plants to resist or tolerate herbivory depends on their functional attributes (Loranger et al. 2012), while the level of impact is related to the growth strategy of the plant and the availability of resources, given that these factors determine the cost of generating new tissue (Reese et al. 2016).

Herbivores influence the nutrient cycling and productivity of an ecosystem through different mechanisms: firstly, they diminish the photosynthetic area of the plants, reducing (in the case of plants that base their anti-herbivory strategy on resistance) the assimilation of carbon and growth (Dubey et al. 2011). While these functions can increase in species tolerant to herbivory, they alter the routes and quantities of carbon and nutrients that are transferred directly from the canopy to the soil, since herbivory reduces the quantity of litter by between 12% and 19% (Hartley and Jones 2004; Metcalfe et al. 2014).

In addition, the deposits of the herbivores (excreta, molted tissues, leaf fragments) act to modify nutrient availability, increasing levels of nitrogen and phosphorus in the soil, due to the fact that the organic material has been physically and chemically fragmented in the intestine of the herbivore and thus decomposes more rapidly than the leaf litter (Fonte and Schowalter 2005; Schowalter et al. 2011; Metcalfe et al. 2014). Finally, it should be noted that the impact of the herbivores on the biogeochemical processes also depends on the functional characteristics of the plants (Díaz et al. 2007; Dubey et al. 2011).

A wide variety of soft functional traits (structural and chemical) have been described as attributes of defense against herbivores. Prominent among these attributes are plant height, trichome density, presence of spines, specific leaf area, leaf density, leaf mechanical resistance, dry material content, leaf nitrogen and phosphorus, carbon-to-nitrogen content ratio (C/N), as well as diverse compounds that are toxic to the herbivores (Díaz et al. 2007; Poorter et al. 2009; Dubey et al. 2011; Loranger et al. 2012). However, few studies have linked the functional responses of the plants to herbivory with its effects on ecosystem processes, despite the fact that this information would help establish a theoretical framework for modelling of nutrient cycling under different herbivory regimes, along environmental gradients and in different scenarios of climatic change (Dubey et al. 2011; Metcalfe et al. 2014; Faucon et al. 2017).

In general, a series of leaf traits, determined by the environmental conditions, have been associated with the intensity of herbivory: species of rapid growth have high values of specific leaf area, high concentrations of leaf nitrogen, low tissue density and cell wall content, high rates of carbon assimilation (assimilated in non-structural compounds such as starches and sugars), and short-lived leaves with high levels of herbivory. In contrast, plants that live in oligotrophic environments present slow rates of growth, prioritizing the retention of nutrients through increased longevity of organs, particularly the leaves, and a high reabsorption of nutrients of the senescent organs. They present high carbon contents in the form of structural compounds, lignin and cellulose, and chemical defenses (Lavorel and Garnier 2002), which makes them less susceptible to herbivory (Coley et al. 1985, Coley 1993, Fine et al. 2004, Poorter and Bongers 2006; Fig. 10.1).

Some of the functional responses of the plants that minimize herbivory are associated with primary productivity and nutrient cycling (Lavorel and Garnier 2002; Reich et al. 2003; Poorter and Bongers 2006; Poorter et al. 2009; de Bello et al. 2010; Dubey et al. 2011; Faucon et al. 2017). This is because they affect soil properties (structure of edaphic communities, physical or chemical properties), regulate processes such as carbon dynamics, increase soil structural stability, and regulate nutrient dynamics, as well as the abundance and diversity of pathogens and microbiota in the soil (Faucon et al. 2017). For example, the hardness, nutritional quality, and chemical defenses of leaves are characteristics that influence their palatability

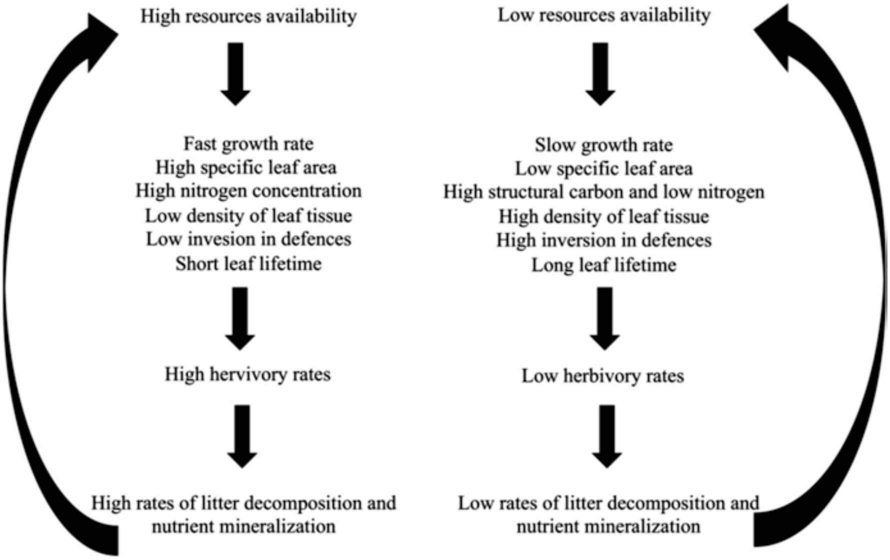

Fig. 10.1 Impact of leaf functional traits on insect herbivory and litter decomposition

and are also determinants of the quality and rate of decomposition of the leaf litter (Dubey et al. 2011). The least palatable leaves present a high investment in structures based on carbon, low leaf nitrogen contents, and high concentrations of defensive compounds (Kursar and Coley 2003). These characteristics are associated with low growth rates in the plants and decomposition rates in the leaf litter (Dubey et al. 2011). In contrast, thin leaves, with high contents of nitrogen and phosphorus and low chemical defenses, which are more susceptible to consumption by the herbivores and are characteristic of plants with rapid growth (Kursar and Coley 2003), increase the quality of the leaf litter and favor rates of decomposition and mineralization. They also promote the development of bacterial and fungal communities, as well as the soil fauna. All of this produces a positive feedback in terms of plant growth (Faucon et al. 2017).

Variation of functional traits is the result of biotic and abiotic pressures and involves correlated characteristics due to inevitable trade-offs (Reich et al. 2003) such as (1) capture vs. conservation of resources (Grime 1979; Tilman 1988; Smith and Huston 1989; Chapin et al. 1993; Poorter and Garnier 1999) or (2) growth vs. reproduction (Silvertown et al. 1993; Solbrig 1993; Reich 2014). For example, a common trade-off is found between the nitrogen content and expansion of the leaf, given that young leaves are more vulnerable to herbivores until they complete their expansion and become harder. Rapid expansion reduces exposure to herbivores, but the leaves become more attractive to the herbivores and suffer greater herbivory as a result due to high nitrogen content and levels of enzymes. In contrast, the species with leaves that expand more slowly present low concentrations of nitrogen.

Although the ideal combination would be one of rapid expansion and low nitrogen content, this is physiologically impossible. With such expansion, high nitrogen content is inevitable and contributes to the high rates of herbivory (Kursar and Coley 2003).

Analysis of the influence of the functional responses of the plants to the herbivores must take into account the fact that these responses are conditioned by environmental restrictions and thus respond in an integral manner to different selective pressures. For this reason, various authors have recently suggested that the convergent expression of characteristics acts as a defense syndrome, with this approach being more appropriate for the study of herbivory and its effects on ecosystem processes (Agrawal and Fishbein 2006). This approach will be detailed in the following section.

Defense Syndromes

In 2006, Agrawal and Fishbein proposed a multivariate approach that considered defense strategies as groups of characteristics or syndromes of defense and defined these as a set of attributes that include aspects of nutritional quality (water and nitrogen content), physical characteristics (spines, trichomes, leaf hardness), toxicity (content of alkaloids and cyanides), phenology, resprouting capacity, and indirect defenses (volatiles and architecture). The predominance of one type of defense over another depends on the cost, benefits, and availability of resources. These attributes interact synergistically, providing a greater level of defense than each one of the attributes could alone, and ultimately maximizes the adaptation of the plants (Núñez-Farfán et al. 2007). However, most studies have considered defenses as unique strategies, with the argument that trade-offs exist between different anti-herbivory defenses, for example, between constitutive and induced defenses (Morris et al. 2006; Cornelissen et al. 2009; Read et al. 2009; Kempel et al. 2011), between physical and chemical defenses (Steward and Keeler 1988; Twigg and Socha 1996; Cornelissen et al. 2009; Read et al. 2009), or between tolerance and resistance (Leimu and Koricheva 2006; Núñez-Farfán et al. 2007). The defense syndrome hypothesis rejects the notion that two types of defenses can be redundant. However, it does assume that unavoidable trade-offs exist among certain functional traits of the plants (Agrawal and Fishbein 2006), for which reason the defenses deployed by the plants will vary as a function of elements of the environment as well as the evolutionary history and intrinsic factors of the plants themselves.

Under the defense syndrome hypothesis, when a group of plants with distant phylogenetic relationships present the same defense syndrome, this could suggest convergent evolution under the same selective pressures. Species that share a common ancestor could be responding in a similar manner due to phylogenetic drag. It is important to note that the syndrome concept does not imply a particular selective agent, but rather the composition and trade-offs of the defensive attributes (Agrawal and Fishbein 2006).

Despite the importance of herbivory in the communities, few studies have linked herbivory with the functional traits of the plants from the perspective of defense syndromes, for which reason it is still unclear which combinations of functional attributes are important to susceptibility to herbivory in the different groups of species and in the different ecosystems and the environments that exist within them (Carmona et al. 2011; Dubey et al. 2011; Loranger et al. 2012; Metcalfe et al. 2014). From a review of the literature from the last 19 years, conducted via an electronic search in ISI Web based on the keywords, defense syndromes, functional characteristics, herbivory by insects, and their combinations, we only found ten publications (Table 10.1) in which herbivory is studied from the perspective of defense syndromes. These publications highlight the following aspects: Firstly, it is notable that the study of defense syndromes is a recent area of research and one that has been little explored to date (with an average of 0.5 articles published per year). One possible explanation for this is the complexity implied by studies conducted at a community level and the evaluation of multiple functional traits. Moreover, with the exception of the study of Moles and collaborators (2013), most of the research has been conducted on few species with many functional traits or on many species with few functional traits. The families Apocynaceae, Brassicaceae, and Pinaceae have received most of the attention. It should be noted that the species of these families present very specific interactions with their herbivores, which acts to restrict any extrapolation of the findings to other systems (Thaler et al. 1999; Fordyce and Malcom 2000; Núñez-Farfán et al. 2007).

One interesting aspect is that of the ten studies, nine report defense syndromes that, in general, correspond to the triangle of defense proposed by Agrawal and Fishbein (2006) (tolerance/escape, nutrition and defense, and low nutritional quality). These syndromes in turn are related to the strategies of resource conservation vs. resource acquisition (e.g., shade tolerant vs. light demanding species or deciduous vs. perennial species) (Table 10.1). These results support the hypothesis that there is a continuum of resource assignation to defense that ranges from species with high levels to those with low levels of defense (Kursar and Coley 2003) and evidences the existence of defense syndromes. It is therefore important that future

Table 10.1 Defense syndromes studies

References	Ecosystem	Study system	Syndrome	Plant traits
Kursar and Coley (2003)	Tropical rain forest	55 species of shade-tolerant trees, shrubs, and lianas	Escape/defense continuum. Rapid leaf expansion and chemical defense	Toughness, nitrogen, chlorophyll, total phenolics, alkaloids, leaf expansion
Agrawal and Fishbein (2006)	Temperate forest (oak forest)	24 species of Apocynaceae	(1) Tolerance/escape, (2) nutrition and defense, and (3) low nutritional quality	Trichome density, latex, toughness, total concentration of carbon and nitrogen, C/N, cardenolide concentrations, specific leaf area (SLA)

(continued)

Table 10.1 (continued)

References	Ecosystem	Study system	Syndrome	Plant traits
Travers-Martin and Müller (2008)	Botanical garden of the University of Würzburg	Seven species of Brassicaceae	(1) High nitrogen content with chemical defenses, (2) mechanical defenses and digestibility reducers, and (3) low nitrogen content and low level of chemical and mechanical defenses	Carbon and nitrogen content, glucosinolates, myrosinase activity and soluble protein concentrations, proteinase inhibitors, trichome density, water content, and specific leaf area (SLA)
Pringle et al. (2011)	Tropical dry forest	19 deciduous species and 11 evergreens	(1) Evergreen species (tough leaves, slow SLA, low water content, and high C/N) and (2) deciduous species (fragile leaves, high SLA, high water content, and low C/N)	Trichome density, latex, toughness, water content, specific leaf area (SLA), C/N, palatability
Da Silva and Batalha (2011)	Cerrado	61 woody species	(1) Tolerance syndrome (low trichomes, latex, chemical and toughness levels, and high nutritional quality) and (2) low nutritional quality (trichomes, toughness and chemicals, high C/N ratios, and low SLA)	Carbon and nitrogen content, water content, and specific leaf area (SLA). Latex content, trichome density, toughness, presence of alkaloid, terpenoids and tannins, carbon and nitrogen content, and C/N ratios
Silva et al. (2015)	Tropical dry forest	Community: three deciduous species and three evergreen species	(1) Drought avoidance (higher SLA, water, and nitrogen contents) and (2) drought tolerance (high leaf thickness and phenolic and PPC concentration)	Thickness, water content, SLA, total phenolics and tannins, nitrogen content
Moreira et al. (2016)	Temperate forest	18 species of Pinaceae	(1) Slow-growing species (high levels of constitutive resin) and (2) fast-growing species (high inducibility of resin and phenolics)	(Concentration of nonvolatile resin and total phenolic) under constitutive JA-induced and SA-induced conditions
Raffa et al. (2017)	Temperate forest	Two species of Pinaceae: *P. contorta* and *P. albicaulis*	(1) Constitutive allocation to compounds to increase resistance and (2) inducibility of inhibitory compounds and allocation to storage compounds	Terpenes, phenolics, carbohydrates, and minerals

(continued)

Table 10.1 (continued)

References	Ecosystem	Study system	Syndrome	Plant traits
Defossez et al. (2018)	Temperate forest	15 species of *Cardamine* (Brassicaceae)	(1) High constitutive defense, high abiotic resistance, and slow growth (high chlorophyll, tough leaves, high constitutive GLS, low inducibility of both GLS and VOCs, and small size plants), (2) high tolerance (high biomass, high C/N ratio, low levels of both mechanical and chemical defenses), and (3) fast resource use for fast growth but low chemical and physical defenses (high stature, high SLA, high inducibility of both GLS and VOCs. and low constitutive defenses)	Plant biomass, plant maximum height, SLA, chlorophyll, toughness, carbon-to-nitrogen ratio, constitutive direct (glucosinolates GSL) and indirect (volatile organic compounds VOCs)

studies consider functional traits that involve the criteria of defense, nutritional quality, and life history of the plants in order to obtain a wider panorama of plant-herbivore interactions.

Perspectives for Herbivory and Plant Functional Diversity Research

While the evidence to date is limited, it is clear that plants respond to herbivory using a set of functional traits that act synergistically and these responses depend on the environment as well as the particular group of plants in question. Furthering this line of research will therefore provide us with information about the responses of the plants under different scenarios of environmental change. For example, the approach of functional diversity and of defense syndromes could contribute to the study of very diverse ecosystems (such as tropical ecosystems), since the integration of groups of species that present similar responses can reduce the complexity of the system, helping to identify the key factors that determine the defensive responses of the plants in highly complex systems (McGill et al. 2006; Díaz et al. 2004, 2007).

Important progress has been made in the characterization of functional traits, as well as the identification of patterns of variation along resource gradients, standardization of measurements, and concepts (Reese et al. 2016). However, further research is necessary in various aspects in order to obtain a more solid conceptual framework for use in different conservation proposals. Prominent among the requirements of such research are:

(a) Identification of trade-offs that limit and define defense syndromes, as well as the use of physiological attributes (Reich et al. 2003).
(b) Studies at different spatial scales in natural systems, since these are the objective of conservation and management (Díaz and Cabido 2001).
(c) Identification of genotypic variation in attributes related to adaptation and holistic studies that evaluate interactions among traits that influence survival, growth, and reproduction in plants and determine how this can generate diversification of attributes in contrasting environments (Ackerly et al. 2000).
(d) Studies that simultaneously evaluate the effects of abiotic factors on different functional traits, at different spatial scales (Díaz and Cabido 2001; Díaz et al. 2004).
(e) Studies of biotic interactions that ultimately determine the ecosystem services that benefit mankind (e.g., pollination, seed dispersion); these studies must consider two or more trophic levels (Díaz et al. 2007; de Bello et al. 2010; Albor et al. 2019).
(f) Studies of the herbivore types (e.g., chewers, suckins, miners, gallers) that could be responsible for the expression of the given defense syndromes (Agrawal and Fishbein 2006; Raffa et al. 2017).

Another line of research is the development of long-term field experiments to evaluate the ecological importance of defense syndromes for the ontogeny of the plants (Travers-Martin and Müller 2008; Moreira et al. 2016; Raffa et al. 2017). It would also be of great interest to understand the deployment of anti-herbivory syndromes at the macroecological level. In this sense, aspects that remain to be addressed include the effect of defense syndromes on the populations of a species that is found immersed in communities of distant composition but in physically similar environments, that is, to evaluate whether selection pressures differ depending on the composition and structure of the communities and to determine the environmental and spatial gradients in the deployment of anti-herbivory syndromes. Development of these themes will allow us to understand the ecological and evolutionary mechanisms that determine the levels of herbivory of the plants at different spatial and temporal scales, as well as to determine their role in ecosystem processes.

Conclusions

Evaluation of the functional responses to herbivory can further our understanding of the impact of herbivores on communities of plants under different environmental conditions; it can therefore help to establish predictive models with which to address processes of global environmental change (habitat fragmentation, exotic species, climatic change, deforestation, etc.). Likewise, integration of these functional traits within the hypothesis of defense syndromes gives us a perspective of the ecology and evolution of plant defenses at the community level, helping to elucidate the associations that exist between communities of herbivores and the adaptive variation of the plants.

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Chapter 11
Natural Herbivore Regulation in Tropical Agroecosystems: Importance of Farming Practices and Landscape Structure

Ana M. Flores-Gutiérrez and Ek del-Val

Abstract This chapter examines the importance of landscape and farming practices in agroecosystems and herbivore regulation in the tropics. It revises their relevance as centers of origin and diversification of agriculture and the threats they experience due to land use change. Here we analyze the patterns that have been observed in terms of management practices that conserve or threat biodiversity and the ones that promote agroecosystem self-regulation, in particular the patterns related to pest regulation and its effects on crop yield. Finally, we discuss the relevance of agroecological studies in the tropics and suggest the use of structural equation models to study, with observational data, the effects of interactions within agroecosystems, the last based on a case study of the effect of management and landscape on the arthropod community in papaya plantations and its cascading effect on plant damage and yield.

Keywords Biological control · Crop production · Insects · Predators · Plant damage

As exposed throughout this book, the interactions between plants and herbivores are very relevant for the evolution and ecology of different natural systems, and in the context of agroecosystems, understanding plant-herbivore dynamics takes on a prominent relevance for management and conservation. An agroecosystem can be defined as a functional system enclosed in a farm unit managed by humans with the aim of providing food, feed, fiber, or fuel where biotic and abiotic components interact (Gliessman 1992). Within agroecosystems the interactions between plants and herbivores are of great concern since they pose one of the greatest problems for production. It is estimated that approximately 18–40% of crop losses can be attributed to herbivore arthropods (Oerke 2006; FAO 2017; Sharma et al. 2017). For this reason, arthropod pests are considered ecosystem disservices (Zhang et al. 2007). In

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contrast, predatory arthropods provide ecosystem services for pest regulation by decreasing arthropod pest populations (Zhang et al. 2007; Rusch et al. 2016).

The diversity and distribution of arthropod communities, herbivores, and their natural enemies depend to a large extent on two significant factors: the characteristics of the landscape surrounding the plantations and the farm management practices. In this chapter, we will particularly focus on tropical landscapes and tropical agroecosystem management and herbivore regulation in agroecosystems, and finally we suggest a novel analysis using structural equation models for the study of observational variables within the complexity of agroecosystems; with this approach, we are able to analyze all the variables in a network and find links between variables not previously contemplated.

Landscape Configuration in Tropical Ecosystems

Tropical ecosystems are immersed in complex circumstances due to cultural, ecological, and political aspects that distinguish them, and therefore it is suggested that tropical ecology deserves a unique subdivision of study and analysis (Vandermeer 2003). Biophysical aspects that characterize them are the lack of a winter season, poor soils, and high biodiversity (Vandermeer 2003) in terms of high species number as well as a high number of endemisms (Laurance et al. 2013). Tropical ecosystems are also situated in a territory with a large cultural diversity; this has implied the evolution of several distinct agricultural managements and the domestication and selection of different crops (Rice 2003).

Unfortunately, in recent times the growing demand for food, extensive population growth, global climate change, and equivocal public policies, as well as the global developmental trends, have caused tropical ecosystems to be constantly threatened by deforestation for their use in economic activities (Laurance et al. 2013). Tropical ecosystems have suffered strong changes in land use since the Green Revolution, for example, from 1960 to 2000, the arable land in Africa and Asia increased in 27%, while in Latin America reached 54% (Song et al. 2018), with a 127% percent of change of area devoted to tropical crops. Along with the increase in area destined for production, there was an increase in yields, which in the tropics was on average twice as much as in 1960 (Song et al. 2018). Another important change that modified the landscape was the use of irrigation; from 1961 to 1998, the percentage of change in irrigated land for developing countries was 100% (Rice 2003). This significant land use change, as well as the management and irrigation, brought important environmental impacts for the world. If we focus on tropics harboring most of the biodiversity, the effects of those changes significantly impacted diversity and ecosystem services (Laurance et al. 2013; Rice 2003; Song et al. 2018).

Even today, the main cause of global deforestation continues to be the expansion of the agricultural frontier in the tropics. The country with the largest area lost is Brazil (with a loss of 380,000 km^2; Song et al. 2018). Furthermore, the most threatened biome with the highest net tree canopy loss is the tropical dry forest (−95,000 km^2, −8; Song et al. 2018). In addition, it is estimated that the land use change will be even more pronounced in the future given that one of the goals for 2050 is to increase global food production by 70–110% (Bruinsma 2009; Laurance et al. 2013) which will have more significant impacts in the tropics (Alexandratos and Bruinsma 2012). It is predicted that the greatest agricultural expansion will occur in South America and sub-Saharan Africa, where areas with little exploitation and high agricultural potential are located (FAO 2009).

With the growing human population, food supply is scarce in some regions, and hunger is experienced by several million people. There are some proposals on how to solve the problem of hunger and, at the same time, produce the food necessary to feed the global population. One is to intensify production in existing crops, so that natural areas that still have primary forest remain preserved (land sparing scenario; Matson and Vitousek 2006), and another is to integrate biodiversity into the agroecosystem (land sharing, wildlife-friendly farming; Tscharntke et al. 2012). Several reviews have proven that in fact the intensification found in the land-sparing scenario is not sustainable, since it brings with it problems that threaten the future of humanity if they are carried out in the tropics (Laurance et al. 2013). Moreover, it does not ensure that deforestation will stop; in fact, there are studies that found that intensification is correlated to greater deforestation (Tscharntke et al. 2012).

Although land sharing has proven to be less damaging for the environment (Perfecto and Vandermeer 2012), this does not mean that there should not be conservation areas associated with this production form or that all the territory should be transformed into agroecosystems (Vandermeer 2003; Tscharntke et al. 2012). In fact, a third scenario that is more consistent with the reality of the tropics is to improve the quality of the agricultural matrix; since currently the tropics are in a state of extreme fragmentation and preserve forested fragments, the approach of promoting small-scale agriculture, such as integral part of the tropical landscape, could preserve biodiversity in a greater extent (Perfecto et al. 2009; Perfecto and Vandermeer 2010).

Tropical Agroecosystems Management

Due to historical and cultural processes, tropical agroecosystems vary greatly in their local and regional landscape configuration and in their farming practices. In these ecosystems, it is possible to find millenary traditional farming practices, subsistence crops, agroforestry and intensive cash crops coexisting (Vandermeer 2003), and farming knowledge exchange.

Traditional Agriculture

Agriculture originated and diversified in certain spots around the world where plants have been domesticated since around 11,000 years ago (Larson et al. 2014). These centers of diversification are found mainly in the tropics and correspond to Southeast Asia, South Asia, East Asia, New Guinea, Africa and Arabia, North America, Mesoamerica, and South America (Larson et al. 2014). Nowadays these diversification centers are still of great relevance for the supply of proteins and calories globally (Khoury et al. 2016).

Traditional forms of agriculture have persisted for hundreds of years, focusing on maintaining long-term production, independent of external inputs (Gliessman 2013), and are based on the use of local renewable resources. Traditional agriculture have also low negative impacts inside and outside the crop, are adapted to local conditions, maintain biological and cultural diversity, are built through the cultural and local knowledge of their inhabitants, and are capable to provide domestic and exportable goods (Gliessman 1992; Gliessman et al. 1981). In this management, there are many indirect practices that prevent pest outbreaks, such as site selection (focused on altitude and type of soil), crop rotation, composition of the surrounding landscape, soil management, intercropping, weed management, harvesting time, and postharvesting management, besides other cultural practices like overplanting to reduce losses (Morales 2002). Additionally, there are direct practices like biological control using domestic birds (i.e., hens), mechanical control using direct trapping, and the use of repellents (Morales 2002).

Conventional Agriculture

According to the USDA, although conventional farming systems are different between farms and countries, they all share characteristics and can be defined as a farming system with rapid technological innovation; large capital investments; large-scale monocrop farms which are grown continuously over many seasons; uniform high-yield hybrid crops; extensive use of pesticides, fertilizers, and external energy inputs; high labor efficiency; and dependence of agro-business (USDA 2007).

After World War II, conventional agriculture expanded throughout the world using new machinery, monocultures, and large quantities of chemical fertilizers and pesticides in order to increase crop yield (FAO 2004). Although crop production significantly increases globally, this type of management also implied massive deforestation and biodiversity loss, particularly in the tropics, and severe environmental degradation and soil impoverishment.

Alternative Agriculture

In response to land degradation, pollution, and human exploitation caused by conventional agriculture, alternative agriculture has been conceived as an agriculture to balance crop yield, long-term soil fertility, and natural pest control using sustainable technologies (Edwards et al. 1990). Alternative agriculture management is based on ecological concepts that enable an optimal organic matter and nutrient recycling, energy closed cycles, balanced populations of arthropods, and polycultures.

Alternative agriculture has become popular because it promotes the use of low-cost inputs and no dependence of external companies, although it also implies high human workforce.

Throughout the development of alternative agriculture, several movements or tendencies have arisen differing on their paradigms, reasoning, and actions (Alviar 2004). We can mention organic agriculture, conservation agriculture, ecological agriculture, biodynamic agriculture, low-input agriculture, permaculture, etc. (Lammerts Van Bueren et al. 2002). In general, organic agriculture is the most known and practiced around the world (FAO 2017).

Organic Agriculture

Organic agriculture is based on sustainable self-regulating production relying on the biodiversity in the agroecosystem, the crops and the associated plants, animals, and microorganisms (Lammerts Van Bueren et al. 2002), where farmers' agrobiodiversity management promotes ecosystem functions, such as pest regulation, pollination, nutrient cycling, and water and soil conservation, to increase resource-use efficiency (Lammerts Van Bueren et al. 2002). For this reason, each organic farmer has different management procedures according to the farm-specific needs; they manage site-specific diversity and the correct assemblages of species to promote ecosystem function. Also, in this type of agriculture, the farmer is himself part of the agrobiodiversity complex. In contrast with the conventional farmer, the organic farmer has to apply, in his own way, ecological knowledge to his specific site situation (Lammerts Van Bueren et al. 2002). At a global scale, some large agro-business are also producing organic products; they apply ecological knowledge to their agriculture, but the production scale is much larger, and sustainability is not always their concern. Organic agroecosystems are characterized by prohibiting the use of chemical pesticides and synthetic fertilizers. Instead they use organic fertilizers, crop rotation, green manures, and mixed farming, which enhance nutrient cycling (Meemken and Qaim 2018).

Influence of Farming Practices on Insect Populations

General Patterns

Crops are highly dependent on ecosystem services of pest regulation, pollination, and soil fertility, which are provided directly or indirectly by insect species (Power 2010; Tscharntke et al. 2012). A reason for this is that the presence of pollinators and natural enemies of pests in crops can lead to a decrease in the requirements of agricultural inputs and, therefore, a decrease in investment costs (Nabhan and Buchmann 1997; Naylor and Erlich 1997).

Farming practices have great influence on insect communities; a meta-analysis (Letourneau et al. 2011) showed that there is less pest damage, less pest abundance, and more natural enemies in diversified agroecosystems than in homogeneous ones. Besides, alternative management systems present a greater biodiversity than conventional systems, both in abundance (50% more) and in species richness (30–40% more; Meemken and Qaim 2018). On the contrary, in crops with conventional management and high use of pesticides, there is a decrease in natural enemy and pollinator abundance (Theiling and Croft 1988; Attwood et al. 2008; Bengtsson et al. 2005; Brittain and Potts 2011). In particular, natural enemy abundance has been associated with their dependence on secondary sources of food like nectar or alternative preys and with refuge or nesting sites, which are usually found in more diverse environments (Bianchi et al. 2006; Chaplin-Kramer et al. 2011; Karp et al. 2018). Coupled with the above, natural enemies tend to be less resistant to pesticides than pests (Theiling and Croft 1988).

It is important to highlight that even when organic agriculture promotes the maintenance of species diversity (Letourneau and Goldstein 2001), this may be due to less intensive management, including several practices of alternative management like use of polycultures, strategies of soil conservation, and fertilization with organic manure, among others (Krebs et al. 1999).

Farming Practices and Yield

Comparisons of crop yield in organic versus conventional systems have found a difference of 25% less production in organic systems (Seufert and Ramankutty 2012). However there are some studies that counter-argue that this gap yield is actually minimum and that systems with polycultures or agroforestry even exceed the production of conventional systems, for example, the polyculture milpa that includes corn, beans, and squash, where corn has a higher yield when it is found in polyculture than in monoculture (Amador and Gliessman 1990). In addition, in contrast with conventional monoculture farming practices, these systems tend to have larger yields during drought years and be more affordable for farmers and less vulnerable to climate shifts (Holt-Giménez et al. 2012; Arnés et al. 2013).

Arthropods and Crop Yield

One of the implications of agricultural management and domestication has been that several plants have lost their defense attributes. A recent meta-analysis showed that plant defense against herbivores in cultivated plants has been negatively selected through human intervention; therefore most cultivars are less defended against herbivores than their wild ancestors, and plant reproduction in agricultural contexts is hampered by herbivore action every year (Whitehead et al. 2017). Therefore, many thousand tons of pesticides are used continuously worldwide to try to prevent this damage with not very consistent results (Karp et al. 2018). The effectiveness of this management strategy is very variable because we are dealing with live systems that imply evolution, and since pesticides are indiscriminately applied, herbivores have become resistant to many of the employed chemicals, and they are not controlled any longer (Roush and McKenzie 1987). Also, herbivore damage is not always directly translated into crop yield losses because plants are able to compensate certain levels of herbivory (Trumble et al. 1993; Poveda et al. 2017); however insecticides are used anyway. Recent investigations highlight the need of a paradigm change regarding crop selection, to aim toward sustainable agriculture and not only to maximize immediate gains (Mitchell et al. 2016). This approach includes to work toward the increase in crop yield but also to focus on the capacity of plants to resist herbivore and pathogen damage in order to decrease externalities caused by insecticides, bactericides, and herbicides and also inevitably in the context of climate change.

Insect herbivores are responsible for important losses in agriculture; some figures propose they can reach up to 40% failure of the total production in some years (FAO 2017). However scientific documentation about the relationship between herbivore abundance and crop yield is not straightforward, mainly because ecological and agronomical disciplines have been separated for long time and quantification of both variables are not considered at the same time in most studies. In particular, ecological studies are generally focused on herbivore fluctuations, and the effect on plant variables is measured on biomass or fitness, but not on crop yield (Letourneau et al. 2011). On the other hand, agronomical studies put their focus on crop yield losses but do not always quantify herbivore abundance. There is a general consensus that herbivores reduce crop yield and that there is a significant correlation between herbivore abundance and reduction in crop yield (Cardinale et al. 2003). However, the relationship between herbivore damage and crop yield is generally not linear; most plants are able to compensate or survive small amounts of damage without repercussions for seed set (Poveda et al. 2003). Therefore, it would be very important for sustainable herbivore pest management to take into consideration the nature of the plant-herbivore interaction in each crop species.

Another important factor to consider for crop production is that not all herbivore damage has the same repercussions on different crops, for example, in experimental cabbage plantations, herbivore effect on crop yield depends upon insect identity and landscape structure, while lepidopteran has less effect when the crop was surrounded by native vegetation, aphids and flea beetles showed the opposite pattern (Perez-Alvarez et al. 2018). One extraordinary example of the opposite effect of herbivore damage upon yield is the herbivory produced by the Guatemalan potato moth (*Tecia solanivora*) on potato (*Solanum tuberosum*) that induced a 2.5-fold increase in marketable potato yield (Poveda et al. 2010).

Pest Control and Natural Enemies

Landscape

Diverse agricultural landscapes may enhance the biological control of insect pests. Landscapes that include natural habitats have been found to benefit natural enemy populations (Andow 1991; Kruess and Tscharntke 1994; Bianchi et al. 2006). For example, in Spain, Jiménez-García et al. (2019) evaluated insect herbivores and natural enemy communities on vineyards surrounded by landscapes with different compositions. They found that land heterogeneity and connectivity with natural areas are an effective strategy for natural enemy conservation for viticulture. Also, for vineyards, Shapira et al. (2018) found that beneficial arthropods are more diverse in natural habitats and that in order to provide better biocontrol, non-crop plants should be conserved inside vineyards and in the surroundings.

In other agricultural contexts, particularly in the tropics, there is less evidence about the importance of landscape configuration for pest regulation (Karp et al. 2018). However, there are studies that have demonstrated the same trends for pest suppression in diversified landscapes in cocoa plantations (Klein et al. 2002), coffee farms (Perfecto and Vandermeer 2002), and rice fields (Settle et al. 1996).

Diversity Inside Plots

The diversification of crop cultivars inside an agricultural plot increases pest control by predators; the mechanisms behind this are related with (1) the failure of pests and herbivores to proliferate sparsely on diversified cultivars because plants have differential susceptibility and (2) predator communities being more diverse in polycultures and therefore exerting a greater biological control (Tooker and Frank 2012). Enhancing diversity in tree orchards also increases biocontrol of herbivore pests in peach farming; Wan et al. (2019) found that natural enemies increased in 38% in diversified plots compared with controls, and these managements translated in herbivore abundance reduction by 16%.

In soybean agriculture, diversified crops varied in volatile signals and had higher insect diversity associated to plants considered as weeds in which the regulation of pests is accomplished by increasing the fauna of beneficial insects and, thus, reducing the use of pesticides (Torcat-Fuentes et al. 2018). However, it is worth noticing the identity of non-crop plants considered to increase diversity in a plot, since some studies have found that in order to decrease herbivore damage on the crop, companion plants should not be closely related with the crop to avoid hosting the same herbivores and to prevent plant competition (Schellhorn and Sork 1997). The services provided by egg parasitoids in rice fields increased in diversified landscapes, but identity of vegetables crops needs to be carefully selected to avoid any potential benefits for rice pests (Vu et al. 2018). In the Philippines, planting string beans in rice fields improves the crop production, but natural enemies or herbivore abundance were not affected; however plots treated with chemical insecticides adversely affected pest regulatory ecosystem functions leading to higher pest damage (Horgan et al. 2017).

Besides the increase in biocontrol, diverse plots are more resilient to extreme meteorological events since the plants also have differential susceptibilities and some are able to produce grain even in bad conditions (Arnés et al. 2013).

Understanding Agroecosystems Complexity Through Structural Equation Models: A Study Case in *Papaya* Crops

As shown in the previous sections, agroecosystems are complex systems in which numerous variables interact and where the relationships are not necessarily direct. So far, we have described the direct effects of management and landscape on herbivory, agro-biodiversity and production, and the multiple cascading effects they have. In a recent review, Karp et al. (2018) found inconclusive results about the relationships between landscape, natural enemies, and pests and concluded that it is necessary to implement models that take into account the complexity of agroecosystems. It is also important to emphasize that the patterns proposed up to now have a bias toward experimental studies and there is lack of observational studies, which could reveal the structural complexity inside the crops (Meemken and Qaim 2018). Considering the above, we identify the approach of structural equation models to be very useful both for analyzing the structure of relationships between variables interacting in agroecosystems and for analyzing nonexperimental data, like observational data taken from the field.

Structural equation models (SEM) are suitable for addressing situations where research variables are complex or multifaceted or to test particular hypotheses about relationships between variables in a network (Lefcheck 2016). SEMs differ from other modeling approaches as they test the direct and indirect effects on pre-assumed causal relationships; this characteristic makes them useful for ecological studies (Fan et al. 2016). The SEM name is related to the study of the structure of relationships through the use of mathematical equations and the possibility to statistically test the data to support or refuse the proposed structure (Fan et al. 2016; Lefcheck 2016). These models are also compared with confirmatory path analysis, models of directed acyclic graphs, analysis of linear structural relationships (LISREL), and causal models (Fan et al. 2016; Lefcheck 2016). The final product of a SEM is a schematic representation of a theoretical model as a path diagram in which we can identify the direct, indirect, and total standardized effects of each variable on the other variables.

In agroecology, when we think about the effects that some variables have on others, many of them are commonly latent variables, which means that they cannot be directly observed and, therefore, are not possible to measure. Examples of these

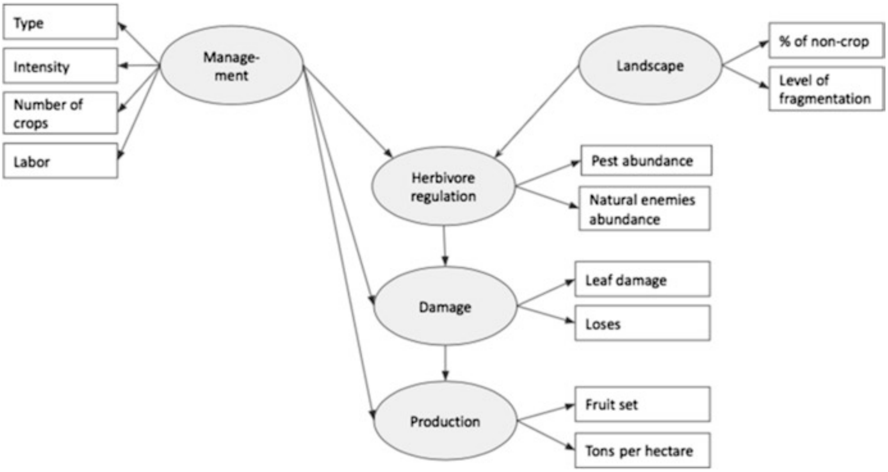

Fig. 11.1 An example of a structural equation model for an agroecosystem. The hypothesis behind is that landscape and management could have an effect on herbivore regulation and therefore a cascading effect on crop damage and crop production. Latent variables are represented in gray circles and their multiple indicators are represented in white boxes

variables can be landscape, management, or biodiversity that can be inferred from observable indicators, such as percentage of forest in a particular matrix, management intensity index, and species abundance, respectively. The main focus of SEM is understanding the complexity of relationships between many latent variables through indicators (Fig. 11.1). As a result of the SEM analysis, each arrow will have an associated correlation value and weight in the whole system, and each variable will have an *r* (Pearson correlation) value; moreover the entire proposed network hypothesis will have a p-value that accepts or rejects the null hypothesis. It is important to notice that our proposed network is the null hypothesis.

Although SEM have a long history, they were hardly used because of the computing requirements for their analysis, which demanded normal distribution of all variables, the need of all variables to be independent, and a high number of observations necessary to fit the model (Grace 2006; Lefcheck 2016). Recent advances, as the ones proposed in the piecewise SEM, are more flexible with the data distribution and the number of observations given that it resolves each equation separately, which allows the fitting of a wider range of distributions and sampling designs (Lefcheck 2016). A difference with previous methods is that this analysis does not incorporate latent variables in the model, but it rather correlates indicators; for this reason they are better called confirmatory path analysis or third-generation SEM (Grace et al. 2010; Lefcheck 2016).

SEM have been used previously in studies of pest regulation in banana crops (Poeydebat et al. 2017) and in studies that evaluate the effects of tropical land use or loss on multiple trophic interactions (Morante-Filho et al. 2016; Barnes et al. 2017). In the case of papaya crops, we analyzed, through the piecewise SEM approach

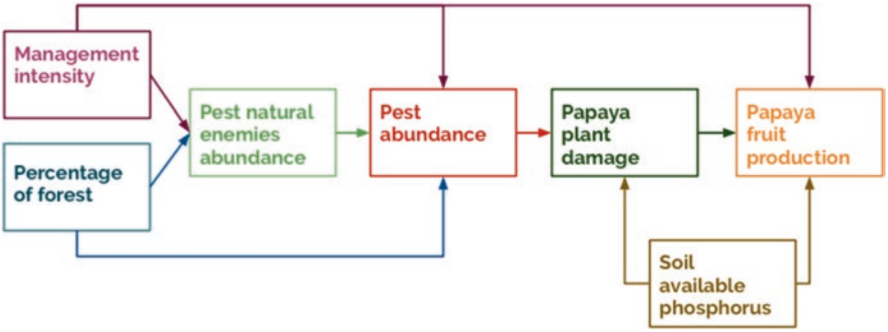

Fig. 11.2 Expected structure of the relationships in papaya crops between their measured indicators (white boxes)

(Lefcheck 2016), how does management intensity, phosphorus soil availability, and percent of natural habitat surrounding papaya plantations influenced arthropod communities, particularly pest and natural enemy abundance, and how these variables could have an indirect effect on crop damage and production in 11 papaya plantations (Fig. 11.2; Flores-Gutiérrez 2019). The relevance of the study was that Mexico is the center of origin of *Carica papaya* and recently native varieties have been substituted for the Cuban variety Maradol. Maradol variety is susceptible to a disease caused by papaya ringspot virus, which is transmitted from sucking herbivores like whiteflies, aphids, and mites. The study was carried in the Pacific Coast of Mexico, in Jalisco state, where papaya crops are expanding and where there are remnants of native tropical dry forest in a mosaic of an agricultural landscape. Given the variety of management practices in the region and the diversity of landscape composition that surround them, it was possible to test the effect of these two variables on herbivore regulation and crop performance through a SEM (Flores-Gutiérrez 2019).

As a result, we were able to identify direct and indirect detrimental effects of intensive management practices on papaya fruit production, characterized for being monocrops with use of synthetic fertilizers and chemical pesticides (Fig. 11.3). Added to the direct effects, the indirect effects were associated with the decrease of natural enemy abundance, but not through the expected herbivore regulation. Instead we found an unexpected direct correlation between natural enemies and fruit production, which appears as a “missing path” in the model analysis. SEM analysis tests the structure of correlations, and as a result, it can lead to reject the proposed hypothetical structure due to significant missing paths. In this case, the explanation of the correlation between the abundance of natural enemies and fruit production might be linked with variables that were not measured, such as their interaction with fruit herbivores or pollinators. Besides, the percentage of forest (Fig. 11.4) was associated only with herbivore pest abundance, which is less in plantations surrounded by forest during the dry season; instead in these plantations, the herbivores increased during rainy season but had no adverse effect on plant damage.

Fig. 11.3 Management practices. The picture on the left shows a polyculture of mango and papaya with an alternative management, whereas the picture on the right shows a papaya monoculture with a conventional management

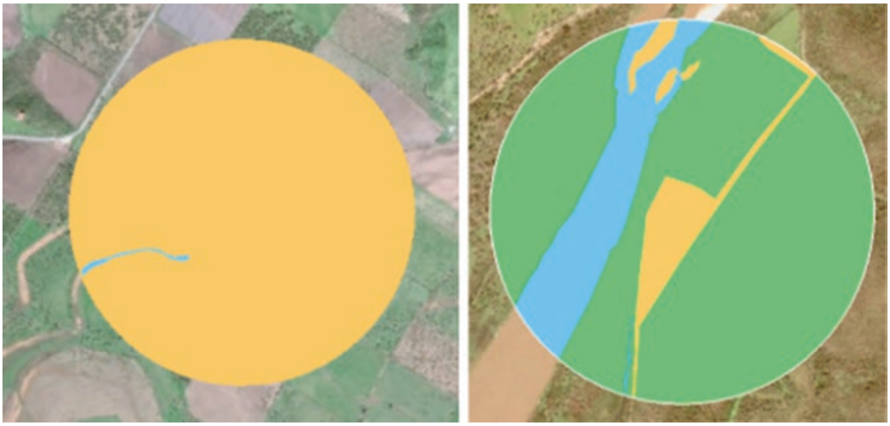

Fig. 11.4 Landscape classification for studied papaya crops. Green = forest cover; yellow = non-forest; blue = water bodies

One of the difficulties of this analysis is the definition of the indicators used and the justification behind the proposed causal relationships, since each proposed relationship can give results that mask intermediate processes that should be taken into consideration. Overall we find this approach is useful not only for data analysis but also for data visualization; SEMs are a great tool to present the results to stakeholders, which, in the end, are the ones who are going to design the public policies or incise in land management.

For our papaya case study, using SEM helped us to conclude that crops with intensive management had less beneficial arthropods and no significant differences in the abundance of pest herbivores, which indicates that the use of pesticides is not decreasing pests, but it is affecting natural enemies. At the landscape scale, a high percentage of surrounding forest was associated with fewer plant herbivores during the dry season and an increase in soil herbivores during the rainy season, with no impact on final papaya production. Therefore, thanks to our SEM approach, we were able to identify from separate and distinct variables that alternative management seems to be a sustainable option for papaya production in the region.

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Chapter 12
Disruption of Plant-Herbivore Interactions in Light of the Current Defaunation Crisis

Rodolfo Dirzo, Roger Guevara, and Eduardo Mendoza

Abstract In this chapter we examine how the current patterns of anthropogenic impact on biodiversity are engendering a pulse of animal life loss – defaunation – with emphasis on the decline and massive extinction of populations of mammals. Given that many species of this group operate as herbivores and, due to their local abundance in some regions and ecosystems of the world, have the potential to affect plant performance and survival, their decline or outright local extinction significantly disrupt the patterns of mammalian herbivory, in some cases causing the local extinction of this critical interaction affecting the structure and composition of communities and ecosystems. Our chapter, addressing a different group of herbivores than that examined in the other chapters of the volume, as well as addressing species interactions in defaunated systems, includes an analysis of mammalian herbivores in the context of insect herbivores, a historical account of the development and evolution of defaunation science, and a discussion of the methods available to document defaunation. (We hope that the inclusion of such contextual analysis and historical and methodological accounts will be of some use for the readers who are not familiar with this field.) This is followed by a brief exposé of the disruption of mammalian herbivory and its consequences at the community and ecosystem levels.

Keywords Global environmental change · Animal population loss · Animal abundance decline · Local extinctions of species interactions · Non-trophic herbivory

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On land, the study of plant-herbivore interactions has been dominated by research that focuses on invertebrates, particularly insects, that operate largely as herbivores that consume leaf tissue or fluids (Dirzo 1984). This emphasis is, naturally, justified given the prevalence of phytophagous insects in the known catalogues of terrestrial biodiversity. Indeed, a nonmolecular-based classification of described species by the late 1990s (Strong et al. 1984; Price 1997; Fig. 12.1a) shows that the so-called higher plants, the main resource base of herbivores, represent 22% of the described species, while the phytophagous insects that feed on them account for 26% of the recognized species. This implies that close to 50% of the known, global species richness of the planet is engaged in the ecological process of herbivory. If, on top of that, one considers the fraction of animals that in addition to being saprophagous are carnivorous insects that have as a feeding habit to consume herbivores, representing an additional 31%, then the food chain of higher plants, herbivorous insects, and their (insect) natural enemies comprises a significant fraction of Earth's known species richness (just shy of 80%) engaged in herbivory. This functional biodiversity perspective also offers evolutionary insights when one further examines the major taxonomic divisions of the Class Insecta, namely, insect orders, and their major functional role (Fig. 12.1b). It is intriguing that out of the 29 or so insect orders, the most speciose ones, namely, Coleoptera (at least 280,000 species), Lepidoptera (not less than 11,200 species), and Hemiptera-Homoptera (minimally 8200 species) are plant eaters. This intriguing pattern provokes the evolutionary speculation that developing the ability to become herbivores probably is not an easy trophic niche to conquer (i.e., only 3/29 orders predominantly do so), and yet, if and when that niche

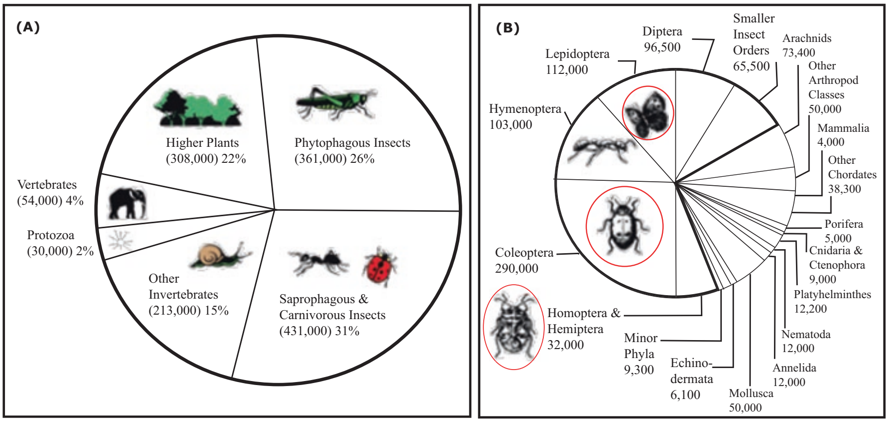

Fig. 12.1 The distribution of the species formally recognized (by year1997) among six major taxonomic/functional groups of organisms, with numbers depicting the percentage (and absolute values in parenthesis) of their corresponding known species richness (**a**), and the distribution of species richness (number of species) in the major groups of the animal kingdom, including the most speciose orders of the Class Insecta (**b**), with the three orders that predominantly phytophagous encircled in red. Panel (**a**) modified from Strong et al. (1984) and Price (1997); panel (**b**) modified from Wilson (1999)

is conquered, this opens the possibility of explosive evolutionary radiation and speciation. This implies that much of the diversification of the planet has been driven by eco-evolutionary relationships between plants and insects. It also implies that the evolutionary ecology of plant-herbivore interactions has been driven to a large extent by insect herbivory.

Beyond insects (and other invertebrates), another group of animals that includes a considerable proportion of herbivorous species is Mammalia. As shown in Fig. 12.1, however, this group dramatically pales in species richness (number recently revised to 5416 species) compared to insects. Comparatively speaking, also, research on mammalian herbivory is also considerably poorer. However, it is well known that in some ecological settings and times, given the local and regional abundance of biomass of these vertebrates, mammalian herbivory has operated as a significant ecological and even evolutionary force. In deep time, the evolution of grasses and grasses' traits has been suggested to be result of evolutionary pressures imposed by mammalian grazers (e.g., Owen and Wiegert 1981). In contemporary times, areas in which large wildlife, including mammalian herbivores, is well conserved, the role of these herbivores on the ecology of plant species, community- and ecosystem-level impacts, and the regulation of ecological cascades have been documented in studies conducted in particular locations (e.g., Dirzo et al. 2014) and in major reviews (e.g., Dirzo et al. 2014; Ripple et al. 2015; Owen-Smith 1988). In addition, we now have compelling evidence that wildlife is undergoing a dramatic and omnipresent decline due to anthropogenic impact – a phenomenon dubbed "anthropocene defaunation" (Dirzo et al. 2014) – and therefore our understanding of the role of mammalian herbivory in light of the current global environmental changes may potentially be a useful complementary contribution to our current understanding of the evolutionary ecology of plant-herbivore interactions, the central concern of the present volume. Some key elements of this chapter (in particular the organisms considered and the disruption of plant-herbivore interactions due to anthropogenic change) are not within the main scope of the volume and it is not expected for readers to necessarily be familiar with the relevant antecedents and methodological approaches for the study of defaunation and its role on plant-herbivore interactions. Therefore, it is our hope that by providing such elements, this chapter may help and ideally stimulate students and young or established researchers to include defaunation in their future research programs. We hope that our review of plant-animal interactions in the context of vertebrate defaunation may provide some insights – or at least some relevant points of reference – for the evolutionary ecology of insect herbivory, given the recent and increasing documentation of the decline of insects around the globe (e.g., Sanchez-Mayo and Wyckhuys 2019) and the ecological consequences of insect collapse.

The History of Defaunation Research

Disambiguation for the Term Defaunation in the Scientific Literature

Although the term defaunation has achieved considerably visibility in the fields of conservation biology and biodiversity science, in order to develop this chapter as part of a book on the evolutionary ecology of plant-herbivore interactions (which, in addition, has a strong focus on insect herbivores), we deemed pertinent to conduct a search of the use of this term in the scientific literature. To our surprise, the use of defaunation (and its derivatives) includes several, somewhat unexpected, connotations. For example, in veterinary and related fields, it refers to the removal of protozoans (and other microorganisms) from the digestive tract of domesticated animals (mostly ruminants) to investigate the workings of symbiotic microorganisms in terms of their effects on nutrient assimilation and consequences for body mass and quality of meat, milk, and fur of domesticated animals (Veira et al. 1981; Sato and Karitani 2009; Santra and Karim 2019). Other scientists have examined symbionts' role in the digestive tract of termites by comparing intact and "defaunated" organisms (Sugio et al. 2006). In marine ecology (1980–2019), defaunated environments are considered those where animals have gone locally extinct, especially due to chemical contamination (e.g., oil spills), or in situations of soft banks where tides periodically wipe out most animals, and the "refaunation" dynamics that follow such perturbations (Santo and Bloom 1980; Faraco and Lana 2003; Sahoo et al. 2017).

Historical Trajectory of Defaunation Studies

Considering the ecological and biodiversity science perspectives of our own research, and the connections thereof for our understanding of plant-herbivore interactions, we offer a brief historical account of the studies leading to the use of the term defaunation, even when some of them did not necessarily use the term per se. Our use of the term can be traced to 1969, when Simberloff and Wilson (1969) published a series of papers describing their results on experimental zoology, whereby following the application of insecticide in six small islands in the Florida Keys to remove the entire community of arthropods, they examined the patterns of colonization. In these elegant articles, the authors used the term defaunation to refer to the (experimental) removal of the animal community. Some years after, Chew (1974) proposed that experimental extirpation of whole faunas from ecosystems would be a necessary experimental manipulation to understand the role of animals in the ecosystem's energy flow and dynamics. A decade later, Janzen and Martin (1982) developed the notion of plant anachronisms to propose that the traits of many neotropical species cannot be fully understood if one does not consider their interaction with the megafauna of the Pleistocene, which must have been an important selective force responsible for the evolution of traits such as hyper-hard fruits or very spiny fruits or trunks. In this and subsequent papers, Janzen argued that the disappearance of the megafauna in the Pleistocene left these plants without their coevolved dispersal or seed predation agents, thus representing neotropical plant anachronisms. Considering this notion from a community-wide perspective, Janzen (1986) argued, for example, that the vegetation structure and functioning of the Chihuahuan Desert "Nopaleras" (*Opuntia*-dominated deserts of Northern Mexico) in areas where domesticated herbivores (cattle, horses, donkeys) are present more realistically reflect the physiognomy and ecology of this ecosystem, compared to areas where these animals are absent. Janzen suggests that those large herbivores brought to the Americas by the Spaniards are the ecological surrogates of the megafauna that used to roam and interact with the nopaleras before the Pleistocene extinction.

Following these pioneer studies, defaunation research experienced some years of dormancy or low visibility, until a book chapter (Dirzo and Miranda 1990) reported a detailed comparative analysis of the abundance and diversity of understory mammalian herbivores from two tropical forests with contrasting degree of conservation. The study also included a thorough documentation of a dramatic reduction in the levels of vertebrate herbivory and understory plant diversity. This study explicitly used the term "contemporary defaunation" in the context of quantitatively linking the current negative impact of the human enterprise on the animal community, leading in turn to correlated alterations of the forest understory diversity and herbivory patterns. The significance of these relationships was boosted by a catchy follow-up review entitled "The Empty Forest" (Redford 1992) which emphasized the fact that in many areas of the tropics, the forest may appear exuberant and seemingly well conserved and yet may be devoid of animal populations and probably undergoing cascading effects on the community of plants and animals. In the subsequent years, our own research has used the term defaunation or contemporary defaunation to quantify the patterns and magnitude of this anthropogenic change at local (e.g., Dirzo et al. 2007; Mendoza and Dirzo 2007) and global (e.g., Dirzo et al. 2014) scales and examine a host of ecological consequences of defaunation (e.g., Camargo-Sanabria et al. 2015; Brocardo et al. 2013), including cascading effects of relevance for human health (e.g., Young et al. 2014).

As indicated above, as vertebrate animals also disappear, a whole lot of plant-animal interactions become disrupted, ranging from energy flow (Chew 1974); plant consumption leading to the deployment of new evolutionary pathways of defenses in the plants, similarly to what happens with invertebrate herbivores (Dirzo 1984); gene flow, thus promoting or maintaining genetic diversity within and among populations (Giombini et al. 2017); fruit dispersal and plant establishment into safe sites (Bagchi et al. 2018); and seed predation affecting the numbers of germinating seeds and subsequent establishment. Through these interactions, vertebrate plant consumers affect plant fitness and demography and may ultimately be key determinants of the composition, structure, and diversity of plant communities (Donoso et al. 2017; Martínez-Ramos et al. 2016). A final point in this historical account is that scientists concerned with past defaunation, such as the Pleistocene extinction of large vertebrates (e.g., Janzen and Martin 1982), have been inspirational to neologists and instrumental for our understanding (and hopefully anticipation) of the consequences of animal extinctions. Initiated before the 1980s (to a large extent championed by our hero, Paul Martin), and with the early 1990s, efforts to link animal declines with the cascading consequences on species interactions, plant communities, and conservation science under the term of contemporary defaunation (Dirzo and Miranda 1990), the following three-plus decades have seen an increasing trajectory of attention to this topic in the scientific literature, with a particularly accelerated trend over the last decade (R. Guevara, in prep.)

Emphases and Biases in Defaunation Research

Despite the importance of all of the ecological roles of vertebrate plant consumers described above, the study of plant-animal interactions in the context of defaunated environments has been clearly uneven, as shown in the word cloud of Fig. 12.2a. Seed dispersal and frugivory are the most studied interactions (33% representation), followed by herbivory and predation (20% and 13%, respectively), while pollination has received the least attention (<4%). Similarly, community-wide ecological processes affected by defaunation show considerable biases (Fig. 12.2b): most studies focus on plant biodiversity (39%) and vegetation regeneration (22%), followed by plant biomass-related aspects (12%), with a lower attention to genetic diversity, carbon storage/sequestering, soil respiration, and decomposition, which collectively add up to 12% of the studies. In terms of geography, an overwhelming majority (80%) of the studies focus on tropical forests (Fig. 12.2c), with a minority of studies examining temperate forests (7%), whereas boreal forest, desert/arid, and

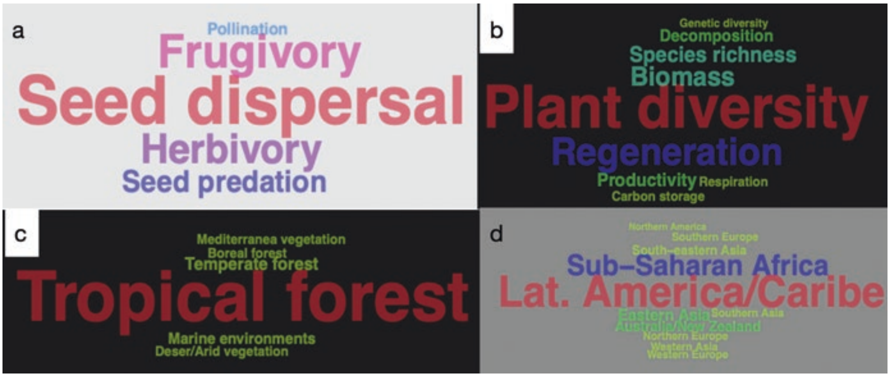

Fig. 12.2 Word clouds depicting the frequency of studies on defaunation concerning (**a**) type of plant-animal interactions, (**b**) response variables at the community level, (**c**) ecosystem types, and (**d**) geographic regions where studies were conducted

Mediterranean vegetation add up to only 8% of the studies and defaunation in the oceans has received the least attention (<4%). Consistent with the geographic and ecosystem-type bias, the most studied region of the world is Latin America and the Caribbean followed by sub-Saharan Africa (Fig. 12.2d). Asia accounts for 16% of the investigations, while Europe has received only a minor effort (8%). Overall, the tropics accumulate 70% of the studies on defaunation, with a neotropical dominance (40%) over the Paleotropics (30%), and only 10% of the studies are in the Palaearctic region. An analysis of the underlying reasons for these biases is outside the scope of this chapter, but it certainly warrants further attention.

How is Defaunation Quantitatively Studied?

The recent years have attested a technological revolution that has impacted most of the aspects of human life. Wildlife monitoring is not the exception, and the study of defaunation has greatly benefited from these developments. Here, we provide a sample of some of technological advancements that have impacted directly defaunation studies.

A central issue in defaunation studies is to assess the impact of anthropogenic activities on animal species' abundance and diversity and in so doing to overcome the perception of human impact on wildlife as an "invisible threat" (Phillips 1996). For decades, the emphasis has been on vertebrates. In their early beginnings, the approaches were detection of the fauna through sightings or the collection of indirect evidence of their presence (e.g., feces and footprints) while walking along transects (Peres 2001; Peres and Nascimento 2006). Whereas the application of these methods allowed generating important information to document the impact of hunting and habitat loss on vertebrate fauna, it has been long recognized that such approaches involve important biases (Tobler et al. 2008; Li et al. 2012). For example, the capacity to observe the fauna and their feces and footprints largely depends on the level of training of the people conducting the survey. Moreover, site characteristics such as soil type and precipitation can clearly influence the likelihood of animal activity leaving reliable signs (e.g., footprints). It was not until the late 1990s that a technological device started to be widely used among the scientific and conservation community to generate better data on the ecology of wildlife (McCallum 2013), camera traps. Although camera trap technology can be traced back to the second half of the nineteenth century, initially its use was restricted to the few people with the skills and resources needed to manipulate the expensive and bulky photographic equipment needed (Kucera and Barrett 2011). It was not only until advances in electronics made it possible to build more compact equipment with greater operative autonomy that the development of very sophisticated camera traps flourished. Since then, the use of camera traps has grown to currently become one of the most widely employed methods in wildlife studies (McCallum 2013). The effectiveness of camera traps is greatly linked to their ability to detect highly secretive fauna due to their low level of intrusiveness and operative independence, allowing them to work continuously over multi-month periods (Caravaggi et al. 2017), thus documenting anthropogenic disturbances on a wide variety of species including those that are very rare and secretive and, therefore, very hard to be detected using other surveying methods (Dinata et al. 2008; Linkie et al. 2013). Furthermore, this technology now allows estimates of population parameters such as abundance and density, by using analyses formerly based on capture-marking-release-recapture (Karanth 1995). Naturally, however, this approach has been limited to animal species where natural body marks (e.g., rosettes, stripes, and scars) allow reliable individual identification (Singh et al. 2010; Karanth and Nichols 2011; Gray and Prum 2012). It is just more recently that methods to estimate vertebrate population density based on camera trapping data without individual identification have emerged (Rowcliffe et al. 2008; Nakashima et al. 2018) – a significant advance for documenting defaunation.

In addition to its high efficiency to record the presence of vertebrate species, camera trapping is particularly well suited to document animal behavior as they perform some of their ecological roles, such as reproductive activity, prey-predator interactions, nest predation, circadian rhythms, foraging, and habitat use (Bridges and Noss 2011). This makes it possible to not only gain an understanding of the impact anthropogenic activity on population and community-level parameters such as abundance, density, species richness, and diversity but also at the functional level. For example, camera traps oriented toward fruits, seeds, or leaves (both at canopy and understory) are very efficient to record, for example, item handling time, plant part selection, etc. (Miura et al. 1997; Prasad et al. 2010; Campos et al. 2012; Rivas-Romero and Soto-Shoender 2015; Camargo-Sanabria and Mendoza 2016). Moreover, particularities of the way animals handle fruits (if they swallow or just eat the pulp and discard the seed) and characteristics of the recorded fauna (e.g., body size) can provide valuable insights regarding seed fate (i.e., dispersal or predation, or both).

Another technique that has gained popularity among ecologists and conservation biologist trying to look at the wider impacts of defaunation is stable isotope analysis (Swan et al. 2020). In particular, the analysis of stable carbon and nitrogen isotopes have been used recently to detect changes in mammal resource and habitat use and trophic structure between preserved areas and human-modified landscapes (Magioli et al. 2019).

In parallel, new molecular techniques have been developed based on DNA retrieved from animal remains (feces, feathers, hair, bone, and scales), yielding a noninvasive genetic record of individuals using genetic markers and offering a way to count and identify individuals in a population, determine their sex and movement patterns, infer parentage or relatedness, and even assess pathogens and diet (Wayne and Morin 2004). Genetic data derived from noninvasive DNA sampling have been used to estimate population density of elusive and endangered animal species, especially in combination with camera trapping data (Kery et al. 2011; Sollmann et al. 2013). A derived asset from the increased availability of global databases of DNA sequences is that this information can be used to generate reference barcode sequences which in turn can constitute an effective tool for monitoring poaching and commercial trade in endangered species (Eaton et al. 2010). Going forward, another technological device that is showing a great potential to monitor wildlife populations is that of unmanned aircraft systems (Linchant et al. 2015).

The Current Patterns of Defaunation

The combination of available technologies up to the recent years, as described above, now permits to paint a picture of defaunation in contemporary times. In order to examine defaunation, it is important to consider that there are three facets that underlie this process, namely, declines in abundance of animals at the local scale, population loss, and global species extinction (Young et al. 2016; Ehrlich et al. in review). The onset of defaunation is human impact on a given species at a given location, leading to a decline in the abundance of animals of the given species. This decline can be driven by direct causes such as overexploitation (e.g., hunting or illegal trading) or indirect causes such as habitat loss or degradation due to land use change (e.g., deforestation, fragmentation, conversion of habitat into human-dominated landscapes, or expansive fires). At the extreme of the impact, such declines eventually lead to the complete obliteration of the local population undergoing human impact. This local population annihilation represents a process of population extinction leading to the species' range contraction. If the loss of local populations expands throughout the species geographical range, it will lead, eventually, to the global extinction of the species concerned. It follows, therefore, that for those endemic species with a very narrow range, and naturally composed of one or few local populations, population loss equates global extinction.

The compilation of information on the status of animals in databases such as the International Union for the Conservation of Nature's Red List of endangered species permits to derive some insights about the magnitude of contemporary defaunation. IUCN's list indicates that, since year 1500, 338 species of vertebrates have become globally extinct. This number increases to 617 if the number of species extinct in the wild (but still existing, e.g., in zoos), and those in high risk of extinction are added. These seemingly low rates of global extinction however, become more meaningful if one considers that a high proportion of those extinctions (197 and 477, respectively) have occurred since year 1900, signaling a dramatic acceleration of global wildlife extinction. Such extinction rates, in fact, are 1000 to 10,000 faster than the known background extinction rates (i.e., those that occur outside mass extinction events). That said, the real defaunation crisis undoubtedly is occurring at the level of population losses. For example, a recent assessment of range contraction in a sample of 177 species of mammals indicates that shy of 50% of the sampled species have now reduced their range in at least 80%, while shy of 20% of the sampled species had reduced their range in up to 20% compared to what it was in 1990. These global assessments are composed of myriad local defaunation events driven by a predominant human activity but more frequently by the combined effects of multiple anthropogenic impacts. For example, overexploitative hunting has been shown to remove as many as 23.5 million vertebrates per year in the Brazilian Amazon (Peres 2000). However, in this and other tropical forests impacted by hunting, this defaunation driver is synergized by the fact that deforestation and fragmentation (a defaunation driver in its own right) facilitate hunters' access to otherwise inaccessible or remote areas.

Finally, another emerging pattern of defaunation is that medium- and large-sized species are much more vulnerable to human impact than smaller species. The former are preferred by hunters, require large areas of habitat to maintain viable populations, and have life history traits, such as low reproductive rates and large generation times, that make them particularly vulnerable, compared to small-sized species (Dirzo et al. 2014). This creates a pattern of differential defaunation, one that seems to be prevalent in areas of anthropogenic impact and one that may have repercussions on the patterns of plant-animal interactions.

These dramatic rates of population loss and local declines in abundance signal that the ecological roles of those animals in "empty forests" have been disrupted and, in some cases, have even led to outright functional extinction. Indeed, it is conceivable that local ecological processes may become locally extinct even before the species has declined to the point of local population extinction (a situation Janzen aptly described as the "living dead"), let alone when the local decline is total. We briefly describe, next, the ecological-evolutionary processes performed by vertebrates and how these are being disrupted by defaunation.

Ecological and Evolutionary Consequences of Defaunation in Terrestrial Ecosystems

The local decline or extinction of medium- and large-bodied vertebrates may have profound effects on plant communities at the proximate and ultimate levels (Galetti and Dirzo 2013), including direct effects on multiple interactions (particularly frugivory and seed dispersal and predation) and herbivory patterns per se and indirect or second- and third-order effects resulting from altered herbivory patterns, including non-trophic herbivory (particularly trampling), as we describe next.

Frugivory and Seed Dispersal

From the newly formed seeds on the plants enclosed in fleshy pulps, defaunation will affect seed dispersion. When large-bodied frugivores, for instance, monkeys and toucans, in wet tropical forests become a limiting factor for the removal of fruits and seed dispersion, mortality rates at the seed and seedling stages are likely to increase. The longer the exposure of fruits/seeds on the plants, the higher the risk of pre-dispersal seed predation, as predators (e.g., insects and birds) are more likely to discover and use these resources (Bonte et al. 2012). Also, nondispersed fruits may remain in the canopy and dry, with null chances for seed germination. However, clumping of seeds and seedlings on the ground around mother trees is the most documented phenomenon caused by the absence of frugivores. When seeds and seedling establish in clumps, particularly below the canopy of parental trees, positive density-dependent mortality is likely to ensue. Clumped seeds and seedlings are more conspicuous and predictable for antagonists such as herbivores, parasites, and predators (Bagchi et al. 2018). Also, limited seed dispersion followed by the impact of herbivores and other natural enemies of plants reduces gene flow (via seeds) that can further jeopardize plant populations in the long term by reducing their genetic diversity and increasing the risk of extinction (Perez-Mendez et al. 2016; Carvalho et al. 2017).

Another piece of evidence on the roles of animals in shaping plant communities and the evolution of their species comes from the Atlantic forest in Eastern Brazil. Galetti et al. (2013) showed that in forest fragments where large frugivores (mainly toucans) are locally extinct, seeds of "palmito" (*Euterpe edulis*) have become smaller than those of sites where all large-sized frugivores are present. The significance of this impact of defaunation on seed dispersal on seedling herbivory is that experimental evidence shows that seed size in some tropical species correlates with the seedling's ability to withstand insect herbivory (Dirzo 1984). Because palmito seed size has high hereditability, populations of defaunated sites will mostly recruit small-seeded individuals. On a broader perspective, Onstein et al. (2018) have shown that palms with large, anachronic megafaunal fruits have either gone extinct or evolved to smaller fruits/seeds during the Quaternary. Recent research at the location of the Atlantic Forest, differential anthropogenic defaunation that has eliminated all relevant top predators has created a predation-release situation whereby small omnivorous monkeys have experienced dramatic population outbursts, leading to an overexploitation of palmito hearts (a food item actively sought out by these monkeys). A multi-year demographic analysis of palmito (Portela and Dirzo, in review) shows that the local populations of this species are undergoing a dramatic decline with Lambda calculations showing that palmito here is decreasing by 34% annually – a prelude to the local extinction of populations of a previously abundant, foundation species of this forest.

Finally, an essentially unexplored ecological interaction linking leaf herbivory and dispersal, the "foliage is the fruit hypothesis" of Janzen (1984) suggests that for many herbaceous species with small seeds or fruits, leaf herbivory by vertebrates implies concurrent fruit/seed consumption. Seeds that successfully transit through the digestive tract of these herbivores can be dispersed to safe sites for germination and establishment. This is an intriguing case of plant vegetative structures serving as the "bait" for dispersal agents whereby the defensive chemistry of leaves and seeds can deploy patterns that would be difficult to explain solely under the optics of leaf defense evolution. This is an aspect that warrants further investigation in light of the omnipresent defaunation affecting understory herbivores in natural ecosystems.

Large Herbivores and Plant Diversity

There is a painful dearth of studies that examine the patterns of mammalian herbivory in defaunated sites, and only a few cases in which individually tagged understory plants are carefully monitored have documented that interaction via comparisons with counterpart not-defaunated sites. In one example, Dirzo and Miranda (1990) examined permanent plots with large samples of individually marked plants to monitor them for evidence of mammalian herbivore scars in a heavily defaunated tropical rainforest site, Los Tuxtlas, and a very conserved site, Montes Azules, both in Southeast Mexico. It is known that historically both sites used to hold the same species composition of understory mammalian herbivores. The results of that study, complemented with a subsequent one, using the same system, are shown in Table 12.1. In the conserved site, it was possible to detect evidence of mammalian herbivory in about 29% of the marked plants in 1990 and in about 24% of the sampled plants in 2003. These estimates are markedly contrasting with the situation of no mammalian damage in the heavily defaunated rainforest site. This dramatic difference signals a situation of mammalian herbivore defaunation leading to the local extinction of an ecological interaction, herbivory.

There is evidence that the extirpation of large herbivores at understory reduces species richness and other diversity metrics of plant communities (Dirzo and Miranda 1990; Camargo-Sanabria et al. 2015). When present, large herbivores not only consume plant foliage in the understory, but they also prey on seeds, particularly large-sized seeds (e.g., Dirzo et al. 2007; Mendoza and Dirzo 2007), and trample on many young/small plants (Rosin et al. 2017). Associated with the cancelation of the impact exerted by large herbivores on plant communities, an additional

Table 12.1 The percentage of plants and leaves bearing evidence of mammalian herbivory in two sites with contrasting levels of understory mammalian conservation: Montes Azules (conserved) and Los Tuxtlas (heavily defaunated)

	Montes Azules		Los Tuxtlas	
				
	Plants	Leaves	Plants	Leaves
(A) 1990				
Seedlings	29.0	30.5	0	0
Saplings (0.5–1.5 m))	30.0	24.0	0	0
Overall	29.3	27.2	0	0
(B) 2003				
Site 1	19.5	–	0	–
Site 2	26.7	–	0	–
Overall	23.5	–	0	–

Plants were examined on two occasions, using plants present in permanent plots of observation in 1990 (A) and an instantaneous measurement of damage in a random sample of 1000 plants in 2003 (B) challenge for the plants seems to come from intra- and interspecific competition which, potentially, may lead to the dominance of competitively superior species. Consumption of seeds and plants by understory herbivores may be selective, avoiding toxic, and otherwise unpalatable, defended species (Simpson et al. 2013). Thus, unpalatable plants may gain an advantage over consumed species as herbivores eliminate many competitors. However, when it comes to large-bodied herbivores, there are positive density-dependent trampling and mortality effects of plants in the understory (Roldan and Simonetti 2001). Thus, strong competitors sensu lato in the plant community, which otherwise would tend to dominate in the composition of the plant community, may be effectively prevented from doing so because of the increased risk of mortality inflicted by large herbivores, via direct consumption or trampling or both (Luskin et al. 2019). Although herbivorous insects may potentially compensate for the loss of large mammals, this is an aspect that hitherto has not been examined.

Experimental studies that have excluded large mammals of plots of tropical rainforests have demonstrated that survivorship of seedlings increased by nearly 20%, and the density of plants increased by up to 65%. At the same time, species diversity decreased by 20% compared to non-excluded plots (Camargo-Sanabria et al. 2015), and predation of large seed is low in defaunated forests compared to non-defaunated forests (Beckman and Muller-Landau 2007). When the exclusion of large mammals reveals no changes in the plant community, it may be an indication that the sites have already lost most of the large-bodied ground herbivores (Brocardo et al. 2013). A different way to look at the importance of large herbivores on communities and ecosystems is through rewilding experiments. For instance, after the reintroduction of bison in experimental plots of mixed grasslands, there was an increment in plant diversity, and this effect was higher than that produced by livestock, despite the fact that bison hardly affected forbs species richness and abundance (McMillan et al. 2019).

In addition to their antagonistic interaction via herbivory and seed predation, some large herbivores also act as long-distance secondary dispersers of seeds, especially of large seeds that withstand the passage through the digestive tract before been deposited long distances away from the point where they initially picked up (O'Farrill et al. 2013). For instance, tapirs effectively disperse seeds of some palm species for which primary dispersion is lacking and seeds accumulate beneath mother plants (Sica et al. 2014). Therefore, a pending research agenda is to parcel the relative impact of mammals as herbivores and seed predators versus dispersal agents.

The long-term and evolutionary consequences in plant communities following the selective defaunation of large herbivores have received little attention. As mentioned earlier, plant communities in the understory may lose diversity because of the expanding dominance of few species. Would these newly dominant species in defaunated sites resemble the behavior of invasive species? What would be the consequence of extreme dominance by few species beyond altering the composition of species of plant communities? Losing plant species with particular traits would likely cascade to affect other ecological interactions that include antagonists (herbivores and pathogens), as well as mutualists (e.g., mycorrhizal fungi and pollinators). Invasive exotic plants may have profound effects in terms of displacement of local species from defaunated sites, given the absence of large herbivores that might serve as biological control agents. The dramatic outburst of exotic species such as *Opuntia* sp. or *Lantana camara* in defaunated savannas may be a reflection of this situation (R. Dirzo, unpub. data). In cases like these, the import of specialist herbivores from the areas of origin of such invasive plants has proven effective in some instances, such as the successful control of prickly pear in Australia, following the introduction of *Cactoblastis cactorum* insect herbivores. Invasive plant species may also affect soil biogeochemistry, mainly altering the nitrogen dynamics (Penuelas et al. 2009) with further negative feedbacks on native species via the disruption of native plant-herbivore interactions.

Carnivores and the Threat to Plant Communities

When large carnivores that operate as top predators are lost from ecosystems, prey species are released from their natural regulating forces, which often bring about demographic explosions of prey populations. In native forests with hyper-abundant herbivores, such as deer and other ungulates, plant recruitment may be seriously limited, and this may have cascading consequences on the plant community. The case of the predation-free monkeys that prey on palm hearts described above is a representative example of this situation in tropical systems (Portela and Dirzo, in review), and the poor recruitment of the foundation oak species is a representative example in temperate and Mediterranean ecosystems (e.g., López-Sánchez et al. 2016). Through browsing and trampling, herbivores may arrest forest regeneration, and if sustained for long periods, the vegetation may be dramatically modified (Asquith et al. 1997). A recent review analyzes the cascading consequences of the loss of top predators in terrestrial and marine systems, including evidence of disruptions of the interactions between herbivores and plants (Estes et al. 2011).

A similar downgrading of trophic cascades has been observed in heavily defaunated locations when top predators, meso-predators, and large herbivores decline or become locally extinct, leading to a release of small-bodied mammals, predominately rodents, which may expand in demographic terms and inflict high pressure on their food resources, such as seeds. Increased seed predation by small mammals, especially predation on medium- and small-sized seeds, will increase, while large seeds will escape predation by rodents (Dirzo et al. 2007). Thus, it may be that in heavily defaunated understories of some tropical forest communities may become dominated by large-seeded plants, unless the recruitment of such large-seeded species becomes prevented by the absence of large-sized frugivores, which seem to be essential for the dispersal and recruitment of such large-seeded species. The teasing apart of the relative importance of these functional groups is a complex research agenda that warrants further examination.

Defaunation in the Oceans

Defaunation is a phenomenon not limited to ecosystems on land. Marine ecosystems are equally under increasing pressure from human activities and changing climatic patterns. Hunting and extirpation of local populations of large predators, whales, and overfishing of small-bodied species are changing animal interactions in the seas (McCauley et al. 2014, 2015). A most dramatic cascading effect of overexploitation of a top predator is that of otter defaunation, leading to the ecological release of herbivorous sea urchins which in turn decimate the individuals and populations of a foundational alga, kelp (*Macrocystis pyrifera*), changing the structure, composition, and dynamics of the emblematic marine kelp forests (see a review of this and other marine defaunation examples in Estes et al. 2011).

Although defaunation in the oceans seems to be a more recent phenomenon than that of terrestrial ecosystems, the rate and extent of damage caused to the oceanic ecosystems has been already considered of planetary concern (McCauley et al. 2015). Recent investigations show that a vast majority of the pelagic fish have experienced a contraction of their distributional ranges (Worm and Tittensor 2011). Adverse effects of defaunation in the sea are likely to extend to land in some terrestrial ecosystems. Sea birds and mammals that bread in large colonies on *terra firma* commonly add a surplus of nutrients to land ecosystems. Marine birds and mammals consume large quantities of fish and mollusks in the sea, and when they settle in their breeding grounds, they deposit enormous quantities of nitrogen and phosphorous (via excrement and urine). Land plants and animals readily exploit this surplus of nutrients, which affect their palatability to invertebrate herbivores, as shown in marine bird-defaunated islets of the Palmyra atoll (Young et al. 2010). Thus, if overexploitation of mass-colony breeders continues, the abundance of nutrients that these animals bring to land ecosystems will be reduced, affecting an enormous diversity of mosses, lichens, bugs, and mites that prosper in the grounds of the breeding colony. Also, plant communities and their ecological interaction will be affected as the footprint of nutrient surplus brought about by sea animals to land ecosystems extends well beyond the colony areas (Bokhorst et al. 2019).

Closing Remarks

Our review indicates that the current pulse of wildlife defaunation has dramatically impacted the diversity, abundance, and composition of species and functional groups of mammalian herbivores. Such defaunation pulse has demonstrable effects on the patterns of ecological interactions between these animals and their plant resources, including herbivory. In some instances, defaunation has led to the decline or even outright extinction of mammalian herbivory. The scant evidence indicates that such disruptions may cascade to a host of processes and interactions within communities, with possible disruptions of the ecological and evolutionary interactions between phytophagous insects and their host plants. Given the increased documentation of the decline of the world's entomofauna (Dirzo et al. 2014; Sanchez-Mayo and Wyckhuys 2019), we hope that this review may throw some light on the nature of the consequences that can be expected under scenarios of insect declines, population losses, and global species extinction and ideally prevent the irreversible loss of the ecological interactions that constitute the architecture of biodiversity.

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Part III
Phylogeny

Chapter 13
The Role of Enemies in Bare and Edaphically Challenging Environments

N. Ivalú Cacho and Patrick J. McIntyre

Abstract We discuss habitat bareness as an integrative factor in the ecology of plants in harsh edaphic environments, with a focus on the role of defense against natural enemies in these habitats. We outline hypotheses related to defense in hostile environments, and present insights from studies that test some of them by integrating phylogeny with ecological observations and experiments on species environments, climate, herbivory, and defense, using a diverse clade of Californian mustards (*Streptanthus* clade) as a system. In this chapter, we (1) discuss "bareness" – the amount of bare ground surrounding plants in a natural setting – as an integrator of the harshness of an environment, with emphasis in edaphic specialization; (2) briefly summarize multiple ways plants defend, resist, or avoid natural enemies; (3) review resource constraints in harsh environments and how these may relate to selection for increased defense in bare hostile environments; and (4) discuss how competition, herbivory, and other biotic interactions may differ in bare and harsh environments.

Keywords Plant defense · Enemies · Resource allocation hypothesis · Serpentine · Glucosinolates · Bareness · Apparency · *Streptanthus* · Crypsis · Edaphic endemism · Stress-gradient hypothesis

Introduction

Habitat specialization is important in generating biodiversity, and plant edaphic specialists represent an important component of both specialization and biodiversity (Cowling et al. 1996; Harrison et al. 2006). A neglected aspect of edaphic

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specialization is its frequent association with bare or sparsely vegetated environments (e.g., a wide range of edaphic habitats are referred to as “balds” or “outcrops”) – in these situations, the intrinsic edaphic challenge may be exacerbated by a lack of plant cover and result in multifaceted challenges related to both soil conditions and the amount of plant cover. The integration of ecology and phylogeny has provided a powerful framework to study the evolution of ecological specialization (Fine et al. 2006; Weber and Agrawal 2012; Cacho and Strauss 2014) and has been central to evaluating the potential importance of bare environments in edaphic specialization. Here we apply insights from this approach to discuss plant ecology in bare and edaphically challenging environments, with a focus on the study of the role of enemies in these conditions.

Environments characterized by sparse vegetation cover result from a wide array of factors, including lack of essential nutrients, physical disturbances such as wind or wave action, harsh temperatures, low precipitation, and chemical toxicity. These environments include warm and polar deserts, talus slopes, serpentine and other chemically peculiar soil barrens, young volcanic soils, beach fronts, and numerous other habitats. Despite the varied nature of bare environments, plants that live in them face common challenges resulting from occurring in a setting with limited vegetation cover (Cacho and Strauss 2014; Sianta and Kay 2019), including the potential for being more apparent to natural enemies, increased UV radiation exposure, vulnerability to desiccation, and mechanical challenges associated with substrate structure and stability. These common factors across bare environments may result in common sets of traits, such as thick leaf cuticles, relatively high investment in defense, and slow growth rates. These suites of adaptations in turn may facilitate ecological and evolutionary transitions from one bare environment to another, as suggested by phylogenetic analyses showing that adaptation to serpentine barrens is associated with lineages previously occupying other bare environments such as rocky bare slopes (Cacho and Strauss 2014). In this chapter we outline a conceptual framework of challenges faced by plants in bare hostile environments and review the mechanisms that plants use to face enemies within these settings. We present insights from the integration of phylogeny and ecology to evaluate some of the hypotheses outlined, including work using a diverse clade of Californian mustards (*Streptanthus* clade) as a model system (Cacho et al. 2014) to evaluate defense in hostile environments.

Section 1. Bareness as an Integrator of the Hostility of an Environment and as a Driver of Soil Specialization

A multitude of factors are involved in making an environment harsh or suitable for plants. One of the main axes in which environments are important for plant life is the edaphic one. A common edaphic factor that imposes harsh conditions for plant growth is nutrient limitation, that is, deficiency in N, P, K, or essential micronutrients. Another important way in which an environment can be challenging for plants is through limited water availability. Water can be unavailable either because of low precipitation and poor soil water retention or due to soil properties such as strong adherence to mineral particles (O'Geen 2013). For example, fine textured soils have low water available to plants despite their large porosity because water is held too strongly to soil particles for plant uptake (below the wilting point, at ≤ -1.5 Mpa) (O'Geen 2013). Ion imbalance in soils is also an important factor to consider, as it can alter nutrient availability; for example, low Ca/Mg ratios can affect a plant's ability to absorb nutrients. Soils can also be high in salinity or toxic elements, especially in heavy metals which sometimes occur in concentrations so high that they challenge all forms of life. In plants, heavy metal toxicity usually translates into reduced germination (e.g., As, Cd, Cr, Hg, Pb, and Zn), reduced growth (e.g., As, Cd, Cr, Co, Mn, Ni, Pb, and Zn), and, in some cases, reduced photosynthetic activity due to direct damage to the photosynthetic pathways (e.g., Zn and Ni). For a review on toxic effects of individual heavy metals, see Chibuike and Obiora (2014).

Other important sources of abiotic stress for plant life are harsh temperatures, which can lower the efficiency of photosynthesis and inflict permanent damage to leaves (Yadav 2009). Harsh temperatures, both low and high, can also be accompanied by high solar radiation. High temperatures elevate levels of evapotranspiration, which coupled with low water availability can act synergistically to impose harsh conditions for plants accentuating drought stress. High levels of solar radiation can also inflict direct damage to the photosynthetic apparatus due to effects of UV radiation (Hollósy 2002). Disturbance, either through fire, erosion, substrate instability (e.g., in steep rocky screes or moving sand dunes), or strong winds, can also contribute to the harshness of an environment. Additionally, physical soil characteristics such as dense barriers or swelling and cracking associated with high clay content can also present mechanical stresses to plants.

Environments are seldom characterized by only a single one of these factors, so harsh environments are usually "multi-challenge habitats." Additionally, edaphic factors can interact with other factors, or be context dependent. A particular soil's texture and water holding capacity can affect nutrient or metal availability, as can a soil's pH (Chibuike and Obiora 2014). For example, in *Thlaspi caerulescens*, low values of soil pH favor Cd and Zn uptake (Wang et al. 2006). Serpentine habitats represent one of the harshest environments for plant life and one in which the edaphic component has been recognized as a major player (Kruckeberg 2002; Brady et al. 2005; Arnold et al. 2016). These habitats are characterized by soils with low nutrient content, low Ca/Mg ratios (which can exacerbate nutrient limitation), and high concentration of phytotoxic heavy metals such as Ni, Cd, and Zn; Ni content in some serpentine soils can reach over 2000 μg/g of soil dry weight (Mengoni et al. 2001). Serpentine environments are also frequently water limited (Kruckeberg 2002; Brady et al. 2005; Cacho and Strauss 2014; Arnold et al. 2016) and often experience high levels of UV radiation, as well as high levels of erosion or disturbance that result in shallow soils (Brady et al. 2005). As a result, areas with serpentine soils are often easy to identify because they present a unique set of species and are noticeably less vegetated than surrounding nonserpentine areas (Whittaker 1954).

The multiple challenges that plants must overcome in harsh environments have each individual effects that translate into slow growth and low reproduction, and we expect that their joint actions would severely affect these two traits related to fitness. Although most attention has focused on abiotic factors, biotic factors also contribute to the varied components to which plants must adapt in harsh environments (see below).

The level of bareness of an environment (the amount of unvegetated space surrounding a plant) has been proposed as a useful integrator of multiple dimensions in which an environment can be harsh for plant life (Strauss and Cacho 2013; Cacho and Strauss 2014; Strauss et al. 2015; Sianta and Kay 2019). Bare environments have, by definition, low vegetation cover due to one or a combination of the aforementioned factors, many of which translate into reduced plant growth and, at the community level, into sparsely vegetated areas. Poor soil quality contributes to low vegetation cover, and these two factors contribute jointly to slow soil regeneration; if the environment also experiences (even moderate) levels of erosion and disturbance, soil quality remains poor or can worsen over time. These factors can also perpetuate (or exacerbate) low water holding capacity and impose a continuous hydric stress for plants occupying these environments. Also contributing to an increased hydric stress for plants growing in bare environments are increased levels of evapotranspiration, which in many environments are a consequence of low levels of shade due to the sparse vegetation cover that is characteristic of these habitats. The effects of having few neighbors are varied and include increased UV exposure, increased apparency to enemies (due to decreased associational resistance; see section 3 of this chapter), and exposure to a less intense competition regime (although, depending on the limiting resource, competition can be more intense as well; see section 4 of this chapter). One important aspect of bare environments is that many of the axes that contribute to their harshness feedback into themselves and interact synergistically in perpetuating or accentuating their hostility (Fig. 13.1).

Traditionally, the study of edaphic specialization focused on comparisons between populations or species (and their soils) that occupy extreme environments (e.g., serpentine barrens) and those occupying nearby or adjacent habitats with more benign soils. Recent studies using serpentine systems as a model, and an ecologically and morphologically diverse clade of Californian mustards (*Streptanthus* s.l., Brassicaceae) in which serpentine specialization has occurred multiple times, evaluated the eco-evolutionary relevance of bareness in the evolution of edaphic specialization focusing instead on comparisons between serpentine and nonserpentine soils of clade mates, with an explicit phylogenetic approach. Soil aspects considered as important agents for selection in serpentine soils, such as a low Ca/Mg ratio or a high Ni concentration, were expected to play an important role in the evolution of serpentine specialists, such that the ability to inhabit soils with such characteristics was expected to evolve jointly with (or precede) the occupation of harsh serpentine environments. The bareness of the microsites in which plants live was compared to the physical (i.e., texture) and chemical (e.g., N, P, K Ca/Mg, Ni) properties of the soils of those same microsites, in the context of evolutionary transitions to serpentine habitats. Microhabitat bareness had a stronger phylogenetic signal than any of

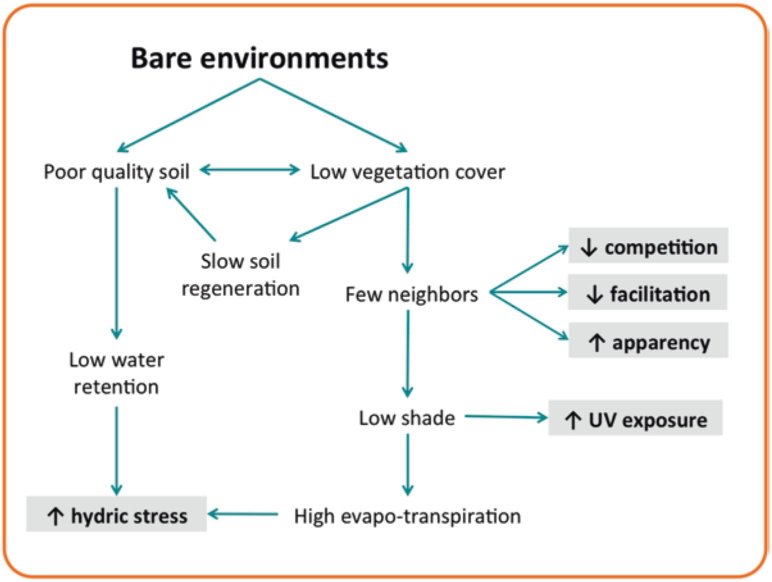

Fig. 13.1 Bare environments are multi-challenge environments in which different factors interact, act synergistically, and feedback into themselves promoting and perpetuating the bareness of the system

the elemental or textural characteristics of soils (Table 13.1), suggesting that the occupation of bare environments has been relevant in the evolution of this clade of serpentine specialists. Analyses evaluating microhabitat field bareness and soil properties in relation to the evolution of serpentine specialization found, as expected, that soil elemental characteristics (e.g., Ni, Ca, Mg, K, P) occurred concomitantly with evolutionary transitions into serpentine habitats. And, contrary to expectation, occupation of bare habitats, but not serpentine soil elemental characteristics, preceded shifts to serpentine. That is, evolutionary transitions toward occupying serpentine soils seem to have happened from ancestrally bare microsites and not from chemically similar soils (e.g., high in Ni or Co, or low in Ca/Mg), as was the expectation (Kruckeberg 1954). This result suggests that adaptation to bare environments (but not soils with similar chemical properties) could have functioned as an exaptation for serpentine habitats and is thus a key factor in promoting serpentine specialization (Cacho and Strauss 2014).

More research is necessary to address the universality of this result. Is there evidence of microhabitat bareness being a precursor of edaphic specialization in clades that have diversified in other edaphically challenging environments such as gypsum or limestone habitats? Can bareness also be a precursor of specialization to harsh conditions other than edaphic? Do we see similar results in other plant groups and more broadly in other systems? A research program investigating these questions necessarily involves phylogenetically centered soil and environment characterizations. Current advances in sequencing technology and phylogenetic methods make

Table 13.1 Phylogenetic signal of bareness and selected soils chemical and textural components calculated over 5000 Bayesian posterior trees of *Streptanthus* and close relatives

Variable	Median K	P-value	Significance
Bareness	1.292	0.009	**
Log (Ca:Mg)	1.151	0.009	**
Log (Co)	0.829	0.009	**
Log (Ni)	0.955	0.009	**
Log (K)	0.949	0.009	**
Log (P)	0.881	0.009	**
Log ($N0_3N$)	0.632	0.149	ns
Log (N)	0.512	0.485	ns
Log (CEC)	0.677	0.030	*
Soil fine fraction	1.443	0.009	**
Soil coarse fraction	1.117	0.009	**
Log (silt)	0.582	0.188	ns
Sand	0.552	0.347	ns
Log (clay)	0.117	0.118	ns

Adapted from Cacho and Strauss (2014)
Phylogenetic signal was estimated using Blomberg's K; K values close to zero indicate no phylogenetic signal, values of $K = 1$ indicate that a given trait evolves along the phylogeny under a Brownian motion model of evolution, and values of $K > 1$ indicate phylogenetic signal stronger than expected under BM. Significance is denoted by one ($P < 0.10$) or two asterisks ($P < 0.01$)
CEC cation exchange capacity

this more of a reachable goal than it has been in the past, and we are optimistic that future research will provide with answers to these questions.

Section 2. Plants Face Their Enemies Through Multiple Mechanisms

Plants use a multitude of strategies and mechanisms to either face their enemies directly (defense/resist; traits that reduce damage), tolerate them (traits that reduce the fitness impacts of a given amount of damage), or avoid them (escape). In this section we provide a brief overview of the topic before focusing on aspects of defense expected to be more important in bare environments. Early literature considered that because some of these strategies are redundant in their overall effect, they should be exclusive and predicted trade-offs between them (Simms and Triplett 1994). The idea was that plants with effective strategies to resist attack would receive little damage and thus would not benefit from investing in tolerance mechanisms and vice versa. However, empirical evidence in the light of more complex scenarios such as diverse herbivore communities and multidimensional constraints has shown that these strategies can in fact be complementary (because defense strategies have different effects on different enemies, among other reasons) and thus be selected for and implemented jointly by plants to face their enemies (Núñez-Farfán et al. 2007; Carmona and Fornoni 2013). Plant defense sensu lato (encompassing resistance, tolerance, and avoidance) is best conceived as a multidimensional space. The study of the evolution of plant defense continues to be subject of active research since the late 1950s (Fraenkel 1959; Ehrlich and Raven 1964; Agrawal and Fishbein 2008; Fornoni 2011; Agrawal et al. 2012; Ramos and Schiestl 2019) and can only be cursorily reviewed here.

The diverse strategies that plants use to face herbivores (and other enemies) include physical (e.g., trichomes, thorns, thick cuticles), chemical (e.g., alkaloids, cardenolides, coumarins, glucosinolates, various kinds of latex), phenological (e.g., timing of young leaf production), ecological (e.g., recruiting enemies of herbivores), and coloration/perception based (crypsis, aposematism) (Fig. 13.2). In addition, tolerance traits include activation of axillary buds, compensatory growth, and resource reallocation within a plant (including roots and storage organs) (Tiffin 2000; Núñez-Farfán et al. 2007; Fornoni 2011; Garcia and Eubanks 2019). Explaining the outstanding variation in abundance, distribution, and diversity of secondary chemistry and other defensive traits has become a field of research in itself but has only rarely been addressed in relation to the bareness or harshness of the environments where defense takes place (but see below).

Ehrlich and Raven (1964) proposed that the evolution of the vast diversity and complexity of plant secondary defenses could have been driven in part by

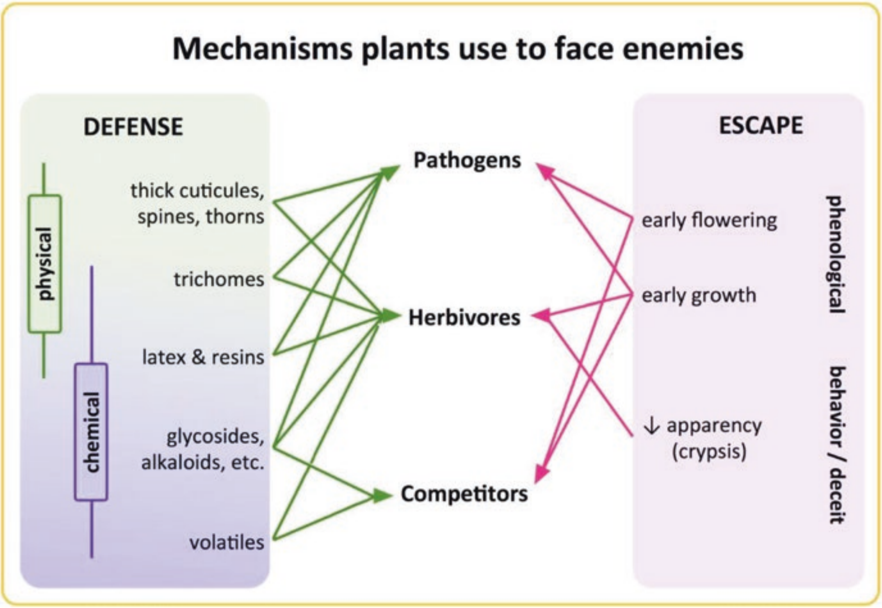

Fig. 13.2 Mechanisms utilized by plants to face enemies are varied, and while traditionally have been classified into resistance, tolerance and avoidance, plants use them simultaneously when facing enemies

coevolution with enemies. Under their hypothesis, plants through secondary chemical defenses have engaged with herbivore counter-defenses in an evolutionary "escape-and-radiate" dynamic that has played an important role in plant diversification associated with secondary chemistry and herbivore counter defenses (Ehrlich and Raven 1964; Berenbaum 1983; Núñez-Farfán and Kariñho-Betancourt 2015). Escalation of plant defense refers to a phenomenon in which sequential cycles of plant-enemy interaction over evolutionary time associated with plant diversification play a role in the evolutionary increase in number, structural or functional complexity, potency, or amount of traits that mediate the interaction between plants and their enemies (Ehrlich and Raven 1964; Berenbaum and Feeny 1981; Vermeij 1994; Becerra et al. 2009). Evidence for evolutionary escalation in plant defense is mixed, and it has rarely been evaluated in the context of the hostility or bareness of an environment. Angular furanocoumarins only occur in relatively few families of angiosperms but are more toxic to specialist *Papilio* lepidopterans than the more widespread and structurally simpler linear furanocoumarins. Richness and complexity in terpene evolution in *Bursera* tend to increase over evolutionary time, in support of the evolutionary escalation of plant defense, but they do so more slowly than species accumulate (Becerra et al. 2009). In *Asclepias*, there is evidence of evolutionary de-escalation in defense, not only in chemical defense (cardenolides and latex) but also in trichomes (Agrawal and Fishbein 2008; Agrawal et al. 2009). In *Streptanthus* and relatives, overall glucosinolate richness, diversity, and chemical complexity decline over evolutionary time, but this is accompanied by an increase in the proportion of aliphatic glucosinolates, suggesting specialization (Cacho et al. 2015). Also in this clade of bare and edaphic specialists, the number of glucosinolates produced by a species increases with the bareness of the microsites plants occupy in the field, supporting microhabitat bareness as an underappreciated selective force acting on plant defense (Cacho et al. 2015).

The study of plant defense placed in an explicit phylogenetic context has provided a strong framework to study adaptive radiation (Becerra 1997; Armbruster et al. 2009), habitat specialization (Fine et al. 2006), ontogenetic changes (Boege et al. 2007; Karinho-Betancourt et al. 2015), relation to breeding systems (Johnson et al. 2010), inbreeding (Bello-Bedoy and Núñez-Farfán 2011), coevolution and arms races with specialist herbivores (Agrawal and Fishbein 2008; Becerra et al. 2009), and the evolution of community structure (Strauss and Agrawal 1999; Agrawal 2007; Becerra 2007; Futuyma and Agrawal 2009; Kursar et al. 2009; Pearse and Hipp 2009), but to our knowledge, the role of bareness has only rarely been considered explicitly in macroevolutionary studies (but see references in section 3 of this chapter related to resource allocation). Other dimensions of plant defense have begun to be explored, namely, within-plant spatial (among plant tissues or organs) and temporal variation in plant defense. The expectation under the optimal defense hypothesis (McKey 1974; Rhoades 1979) with respect to within-plant spatial variation in defense is that defense will be concentrated in tissues that are of higher fitness value to the plant. In agreement with this expectation, *Boechera stricta* fruits are more defended than leaves (Keith and Mitchell-Olds 2017). It would be interesting to consider whether the difference in defense among a plant's tissues or organs is exacerbated in relation to the bareness or hostility of an environment, as the resource availability hypothesis would predict (see section 3 of this chapter). Temporal variation in the activation of defense mechanisms has also proven to be effective: the performance of the herbivore *Trichoplusia ni* was reduced by temporal variation in defense in a way that is more than the average of its parts (Pearse et al. 2018). How temporal variation in defense is related to habitat hostility is a venue that is worth exploring: Does the harshness of an environment amplify or diminish the effects of temporal variation in defense? Of special interest are exciting venues addressing the joint effects of pollinators and herbivores on plant traits and defense. Using rapid cycling *Brassica rapa*, Ramos and Schiestl (2019) show a significant effect of the interaction of herbivory and pollination in the production of chemical defense. Again, it would be of interest to evaluate the behavior of the interaction in relation to the harshness/bareness of the environment in which it takes place.

In the following sections, we discuss how habitat bareness, by potentially influencing exposure to enemies, imposing constraints on growth, and altering selection on secondary chemistry, poses challenges that may influence plant investment in defense, and how defense strategies may trade off with other constraints and challenges in harsh environments.

Section 3. Hostile Environments Select for Increased Investment in Defense

Multiple factors contribute to making bare environments with poor-quality soils hostile for plant life. The resource availability hypothesis (RAH) and apparency theory are useful frameworks to place the expectations of the joint effects of the multiple factors of promoting harshness of bare environments. Below, we outline how, due to a combination of factors (including those outlined in section 1 of this chapter and Figs. 13.1 and 13.3), we expect selection for increased investment in defense in plants (and other organisms) inhabiting hostile bare environments in the context of the RAH and apparency theory.

The RAH explicitly recognizes that the context in which defense takes place is of utmost importance (Janzen 1974; Coley et al. 1985; Fine et al. 2006), and it directly incorporates the types of habitats in which plants grow to make predictions related to investment in defense. The RAH postulates that in environments where tissue replacement is costly (such as bare hostile environments) and growth occurs at a rather slow rate, plants will likely, rather than invest in tolerating herbivory, exhibit an increased investment in defense to protect expensive tissues (Janzen 1974; Coley et al. 1985; Fine et al. 2006). Resources are thus allocated to defense rather than growth, although integrating the cost of defense is also necessary and important. Predictions of the RAH for bare environments are thus straightforward (Fig. 13.3): plants living in bare environments that impose high costs of tissue replacement are expected to invest more in defense (Janzen 1974; Coley et al. 1985;

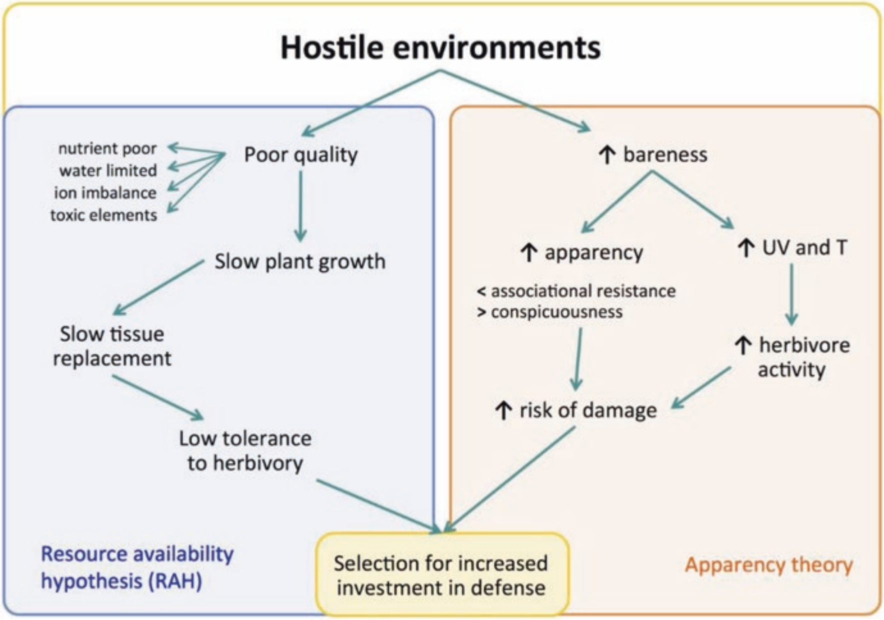

Fig. 13.3 Hostile environments are usually characterized by bareness and poor-quality soils, which are related and interact. The resource availability hypothesis and apparency theory are useful frameworks to place the expectations of the joint effects of the multiple factors of promoting harshness of bare environments

Fine et al. 2006) than close relatives or similar plants inhabiting more benign areas. In a now classical study where species from six genera across five plant families of tropical Amazonian trees native to clay soils and their congeners native to sandy soils were reciprocally transplanted, Fine et al. (2006) showed that species from nutrient-rich clay soils invested less in defense and experienced higher herbivory with fitness consequences when transplanted into nutrient-poor sandy soils. Also in agreement with predictions of the RAH, glucosinolate richness in species of Californian *Streptanthus* is correlated with microhabitat bareness in phylogenetically explicit analyses of defense in bare environments, and 10 of 14 relationships between specific glucosinolate classes and nutrient availability were negative (Cacho et al. 2015).

The probability of a plant being attacked by herbivores has also been shown to be related to its apparency, defined as the ease with which plants can be found by searching enemies (Feeny 1976; Rhoades and Cates 1976; Barbosa et al. 2009). Apparency is thus another force that is thought to be involved in shaping plant defense and has the potential to play a strong role in sparsely vegetated habitats (Feeny 1976; Rhoades and Cates 1976; Strauss et al. 2015). Neighbors, among other things, can decrease the ease with which a plant is located and attacked by physically or chemically “obscuring” it, a phenomenon recognized as associational resistance (Karban 2007; Barbosa et al. 2009; Castagneyrol et al. 2013). The sparse vegetation that characterizes bare environments makes them simple searching environments where associational resistance is – by definition – lower, and thus apparency and encounter rates with enemies are expected to be higher and impose a stronger selective regime for increased investment in defense, than in more vegetated habitats (Figs. 13.3 and 13.4).

A recent review and work within the *Streptanthus* system suggests the following predictions from apparency theory regarding plant defense in simple searching bare environments (Strauss and Cacho 2013; Strauss et al. 2015):

1. Plants in bare environments should be more defended than plants inhabiting more vegetated environments.
2. Plant defenses that rely on signals/perception/detection (e.g., aposematism or hiding through crypsis) should be more frequent in bare environments than in environments with more vegetation cover.
3. Greater variance in damage for cryptic/aposematic species, which are expected to experience less damage as a result of their protection (i.e., high proportion of zero damage), but, if detected by enemies, experience more damage (because they are more palatable than more defended apparent plants).
4. Plant defense and plant apparency should covary: more apparent plants should defend more (chemically or physically), while plants that defend through crypsis might be more palatable (and if found, more damaged than unpalatable apparent plants).

Multiple lines of evidence offer support for these expectations. In plants naturally occurring in serpentine bare environments, field surveys revealed that microhabitat bareness was positively correlated with damage inflicted by herbivores (Strauss and Cacho 2013). Field manipulations revealed higher herbivore attack rates in plants whose apparency was artificially increased by removing surrounding vegetation in serpentine outcrops but not in surrounding more vegetated areas (Strauss and Cacho 2013). Crypsis, or background matching, was also documented as a mechanism of defense in *Streptanthus*: naturally occurring plants that were experimentally mismatched from their background by adding sieved soil or sand suffered significantly higher rates of damage than controls, consistent with crypsis providing defense against herbivores (Strauss and Cacho 2013). In New Zealand scree habitats, scree-matching species had greater variance in damage than conspicuous species (Strauss et al. 2015). Similar observations have been made at the community scale, in strandline (highly heterogeneous habitats with sand or cobble stones as substrates) plant communities of Narragansett Bay, Rhode Island, and Massachusetts: removing all neighbors but keeping herbivores led to a significant reduction of plant biomass (% cover) likely due to increased herbivory (Heard and Sax 2013), which is in agreement with associational resistance being offered by neighbors.

Fig. 13.4 Barren habitats are characterized by multiple stressors, and plants face them, including enemies, through a variety of strategies. Clockwise: (**a**) Serpentine habitats are often embedded in a matrix of more benign substrates and are easy to identify because they sustain a more sparse vegetation characterized by a different set of species from surrounding areas. (**b**) Bareness is not exclusive to peculiar soils environments. *Caulanthus inflatus* is a nonserpentine species that occurs in somewhat bare environments. (**c**) *Streptanthus callistus* occurs in nonserpentine but very bare, rocky environments. (**d**) *Streptanthus morrisonii*, a serpentine apparent species, is highly defended, through chemical and physical (thick cuticles) mechanisms. (**e**) *Caulanthus amplexicaulis* is a nonserpentine species which matches its background, decreasing its apparency despite the lack of neighbors. (All pictures and artwork by N.I.C)

Section 4. Beyond Defense: Multiple Types of Interactions in Harsh Bare Environments – Stress Gradients and Multiple Interactions

Interactions in Harsh Environments: The Stress-Gradient Hypothesis

Current ideas regarding the strength of ecological interactions in harsh environments are probably best encapsulated by debates surrounding the stress-gradient hypothesis (Callaway et al. 2002). This hypothesis builds on Grime's (Grime 1977, 1979) ideas about the role of competition along stress gradients to incorporate the observation that as the abiotic environment becomes more stressful, interactions among plants may shift from competitive to facilitative or mutualistic because amelioration of abiotic stress becomes a key factor. Although this basic version of the stress-gradient hypothesis has attracted support (He et al. 2013), findings that competition may increase with stress have led to refinements focused on complexity related to the severity of stress gradients and the nature of stresses such as the availability of limiting resources (e.g., water scarcity or nutrient limitation) versus non-resource stresses (e.g., alpine wind shear, harmful UV radiation, soil disturbance) (Maestre et al. 2006, 2009). Additionally, the need to consider the importance of multiple types of interactions alone and in combination (e.g., resource competition, herbivory, competition for enemy-free space, pollination, and the role of predation and trophic cascades) complicates the question of how the relative importance of ecological interactions such as competition, facilitation, or herbivory change as environments become harsher.

Bare environments in many cases represent extremes of stress, highlighting the issue of considering interactions not only in challenging conditions but at environmental extremes limiting to growth (e.g., Michalet et al. 2014). The question of whether interaction importance across a stress gradient is characterized by a monotonic (generally increasing or decreasing), unimodal (hump shaped), or more complex relationships is a frequently addressed modification of the stress-gradient hypothesis and is particularly important at extremes of stress (Maestre et al. 2006; Michalet et al. 2006; Qi et al. 2018). To reconcile conflicting support for the stress-gradient hypothesis, Maestre et al. (2009) presented a conceptual model where the nature of interactions with stress depends on the type of stress. In cases where extreme stress is associated with a physical challenge such as wind, interactions may continue to be positive even at the most extreme levels of stress. In contrast, where stress is also associated with a limiting resource such as water or nutrients, interactions that originally were more positive owing to stress amelioration may shift back toward competitive, as the ability to compete for a scare resource becomes more limiting than environmental amelioration. This scenario is complicated further in bare environments, where multiple stressors coexist and interact, often acting synergistically (addressed in section 1 of this chapter).

Fitting Bare Environments into the Stress-Gradient Context: A Case for the Importance of Multiple Interactions

Bare environments, such as serpentine barrens and many other sparsely vegetated edaphic habitats, often represent islands of low vegetation cover within a matrix of more densely vegetated areas. Thus, the general regions in which these bare habitats occur are characterized by temperature and precipitation conditions that typically support higher productivity, in contrast to sparsely vegetated alpine, polar, and hot desert environments where strong abiotic stresses are ubiquitous in the broader environment. In this context, bare edaphic habitats do not fit neatly into a simple stress-gradient framework, but may instead highlight the importance of multiple constraints and complex interactions among the environment and community members. Models to explain adaptation to bareness in serpentine habitats often invoke trade-offs between growth and serpentine tolerance, interactions with neighbors beyond competition, or synergistic effects of multiple abiotic and biotic stressors in combination with ecological and evolutionary trade-offs (Harrison and Rajakaruna 2011; Sianta and Kay 2019).

Results from experimental and descriptive approaches assessing the effects of neighbors in two *Streptanthus* species endemic to serpentine environments reveal that the net effect of neighbors in the field, measured over their lifetime, was negative (Strauss and Cacho 2013). A positive effect of neighbors that reduced herbivory (through associational resistance) was not enough to compensate for the negative effects of competition in this example. It may be that in bare serpentine environments, opportunities for facilitation through environmental amelioration are limited relative to the consequences of competing for limiting resources (e.g., water, nutrients) in tandem with physiological challenges related to withstand low Ca/Mg ratios or high concentrations of heavy metals. At the same time, opportunities for facilitation or indirect competition through interactions with other species may be greater in these habitats (e.g., if herbivores, pollinators, pathogens, and predators are not themselves limited directly by edaphic factors but indirectly through plant abundance and habitat bareness). Relative to strong abiotic resource limitation or physical stress, these biotic interactions may be more temporally variable.

The role of competition and herbivory may also be influenced by ecological and evolutionary feedbacks related to competition and stress tolerance in serpentine endemic *Streptanthus*. In experiments with an explicit phylogenetic design, Cacho and Strauss (2014) showed that taxa from bare environments are poorer competitors than clade mates that inhabit more vegetated areas. They measured the log response ratio (lnRR) in lath-house experiments performed on field collected soils and related it to microhabitat bareness measured in the field, using an experimental design and models that explicitly incorporate phylogenetic relatedness. They found that competitive ability is negatively related to field microhabitat bareness, in agreement with the notion that edaphic specialists are confined to harsh substrates through competitive exclusion because the adaptations that allow them to cope with harsh conditions come at a cost of competitive ability (competitive exclusion paradigm) (Salisbury 1921; Kruckeberg 1951, 1954; Baskin and Baskin 1988). Recently, Sianta and Kay (2019) found similar results in a broader phylogenetic context. An additional modification of the stress-gradient hypothesis suggests that species that emphasize a stress tolerant rather than competitive strategy would be associated with negative effects of neighbors in stressful environments (Maestre et al. 2009). Evolutionary trade-offs in competitive ability could be the result of the combined effect of multiple sources of selection acting in harsh, bare environments (see section 1 of this chapter on bare environments) and may make negative effects of neighbors more likely relative to positive effects.

Future Directions

Integrating and understanding the role of multiple types of interactions acting simultaneously in harsh bare environments is a key area for investigation. Although a number of studies and models have looked at interactions between competition and facilitation, and the role of apparency to natural enemies (Smit et al. 2007; Crain 2008; Graff and Aguiar 2011; Strauss and Cacho 2013; Louthan et al. 2014), clear patterns of the importance of apparency and associational resistance in relation to stress have yet to emerge. Predictors of situations in which the effects of herbivory and apparency with stress may override relationships between competition and stress have yet to be identified.

A deeper understanding of the relationship between plant-plant and plant-herbivore interactions in harsh environments is needed, as well as investigation into other types of interactions in harsh environments and across gradients of plant stress. Integration of the dynamics of facultative mutualists is also key, as interactions between plants and endophytes or rhizobia, for example, may shift from positive to negative depending on the presence of herbivores or the availability of water (mutualism-parasitism continuum). Interactions with pathogens and pollinators are likely to be complex and may depend on whether interacting species are structured by stress and plant density at a similar scale as the plant community. Lever et al. (2014) provides an example of extending the stress-gradient hypothesis to pollinators in highly nested communities to suggest a role of nestedness in containing community collapse, and it would be interesting to further extend community network frameworks in the context of harsh multi-challenge bare environments.

We advocate that research along these and other venues includes the integration of field observation and experimentation, both in phylogenetic and community contexts. An explicit phylogenetic approach and integrating scales other than the focal species are crucial to gain a clearer picture of the importance of interactions in the ecology and evolution of plant life in multifaceted harsh bare environments.

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Chapter 14
Evolution Among Weevils and Their Host Plants: Interaction Between the Genera *Trichobaris* Le Conte and *Datura* L.

Marisol De-la-Mora Curiel

Abstract To understand the diveregence of herbivorous insects associated to plants, in terms of speciation, is useful to consider the evolutionary trends of the species, the geography and hystoricity of insect-plant interactions. In this chapter I will point out the major hypothesis in the speciation of herbivorous insects and describe a more integrative view of their speciation process, as an example, I will describe the results that I had obtained in the study of evolution of the weevils of the genus *Trichobaris* using several approaches, such as geometric morphometric, phylogenetics, and phylogeography. Finally, I conclude describing the trend in the evolution of this weevils.

Keywords Weevils · Speciation · *Datura* · *Trichobaris* · Host plant · Phylogeography · Phylogeny · Geometric morphometric · COI · Rostrum · Insect evolution

How Plants Promote Speciation in Herbivorous Insects?

The main hypothesis to explain the biodiversity of herbivorous insects implies that plants might play an active and major role in the ecological speciation of insects (Ehrlich and Raven 1964). This is especially true in Lepidoptera and Coleoptera groups, which are very evolutionary successful groups. In fact, they have the greatest number of described species and present such diversity that occupies almost all terrestrial niches (Grimaldi and Engel 2005).

The hypothesis stands "Angiosperms have through occasional mutations and recombination, produced a series of chemical compounds […], these compounds,

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by chance, serve to reduce or destroy the palatability of the plant […], such plant protected from the attacks of phytophagous animals, would in a sense have entered in a new adaptive zone. Evolutionary radiation of the plant might follow, and eventually what began as a chance mutation or recombination might characterize an entire family or group of related families. […] If a recombinant or mutation appeared in a population of insects that enabled the individuals to feed on some previously protected plant group, selection could carry the line into a new adaptive zone. Here it would be free to diversify largely in the absence of competition from others phytophagous animals" (Ehrlich and Raven 1964).

This hypothesis is based on the main assumption that specialization on plants brings insect speciation and goes from the microevolutionary level to explain macroevolutionary level. This brings a framework to study insect-plant interactions, starting with the modification of the traits that are mediating the interaction, followed for a population level differentiation, and ending with the origin and of independent evolutionary lineages. Nonetheless, there are few documented cases where the congruence among phylogenies of insects and plants can be found (Jousselin et al. 2008; Percy et al. 2004). It happens because the interaction of this groups with angiosperms is more complex than it was thought. Insects, like other organisms, are influenced by microevolutionary factors such as mutation, genetic drift, gene flow, and selection. Multiples sources of selection can be promoting speciation, including divergence in plant preference, predation (cryptic morphology), mating preference, intraspecific competition, etc. (e.g., Supple et al. 2014; Matsubayashi et al. 2010). In some cases speciation may occur via genetic drift mailny, instead of selection; it is due to founder events and population bottlenecks, especially on insects with patchy distributions and small local population sizes caused by host plant shifts or because they have recently colonized islands and do not possess high dispersal abilities (Matsubayashi et al. 2010; Roderick 1996; Roderick and Gillespie 1998).

The knowledge of historicity of the interaction as well as the geography of the interaction is fundamental to understand the relationship between insects and plants (Futuyma and Agrawal 2009; Farrell et al. 1992; Thompson 2005). The geographical genetic variation of the species involved allows to estimate the role of microevolutionary factors (gene flow, genetic drift, etc.) in the process of population differentiation. Thus, phylogeography facilitates the understanding of the complicated roles of geography associated with population structure, genetic breaks due to barriers to gene flow (Marske et al. 2013) and how genetic variation associated with plants is found (e.g., Hernández-Vera et al. 2010).

Evolution of Weevils and Role of Their Host Plants

The family Curculionidae (Coleoptera), commonly known as "the true weevils," is one of the most diverse group of herbivorous insects with around 360,000 species (Grimaldi and Engel 2005). Phylogenetic approaches show that the diversification patterns of flowering plants and weevils present temporal lags between these groups, but increases in the diversification of plants are followed by an increase in the weevil diversity (McKenna et al. 2009). Then the co-evolutionary process is more complex than straightforward cospeciation, the temporal lags can reflect specialization in plant tissues. For example, most of the weevil's larvae develops on plant tissues: stems, leaves, roots, flowers, and/or fruits (Marvaldi et al. 2002). Diversification of insects may have occurred in plant tissues but, studies among weevils and plant genera are scared to test this hypothesis, usually the knowledge of the host plant is poorly known (just family name) or the genera of the weevils are not known (Anderson 1993).

Phylogeographical studies show different patterns of the distribution of genetic variation of weevils given the tissue where they oviposit (Hernández-Vera et al. 2010; Toju et al. 2011; Aoki et al. 2011). The species of weevils that oviposit into the fruit of a host plant have the same phylogeographical pattern that the one of the host plant, but the weevils that oviposit on leaves have a different phylogeographical pattern that of the one of the host plant.

The key traits of the evolution of weevils are related with the success of oviposition. This traits are endophagus larvae, a long mouth (rostrum), and bimaculate antennae. Most of these insects dig a hole with their mouth to oviposit into the plant tissue, and the bimaculate antenna is bent to dig deeper (Oberprieler et al. 2007).

Study Case

To study the evolution of weevils and determinate how the plants influence its evolution, we focused on the evolution of *Trichobaris* genus and their host plant. There are 12 described species in the genus: *Trichobaris major* Barber 1935, *T. soror* Champion 1909, *T. pueblana* Casey 1920, *T. trinotata* Say 1831, *T. insolita* Casey 1892, *T. championi* Barber 1935, *T. mucorea* Le Conte 1854, *T. bridwelli* Barber 1935, *T. compacta* Casey 1892, *T. pellicea* Boheman 1844, *T. texana* Le Conte 1876, and *T. cylindrica* Casey 1892. Six of these species develop into fruits of *Datura* species, three species into the stems of potato, tobacco, and tomatillo (*Solanum tuberosum*, *Nicotiana attenuata*, and *Physalis* sp., respectively) and four into the stems of wild species of *Solanum* (*S. eleagnifolium*, *S. rostratum*, *S. carolinense*) (Barber 1935). The life cycle of these weevils is closely associated with their host plants (Cabrales-Vargas 1991), for instance, larvae of *T. bridwelli* and *T. compacta* cannot survive in a different host (Cuda and Burke 1991; Lee et al. 2016). Ever since Barber's (1935) monographic study, the genus has not been studied again in such depth. His work on *Trichobaris* includes specimens from a wide range of locations and detailed morphological descriptions. Nevertheless, Barber (1935) pointed out issues not yet resolved, including the need of precise information on host plants and the relevance of body shape and size in delimiting morphological species because some named species were thought to be environmentally related variants. Considering the phylogenetic relationships among these weevils, we tried to find correspondences with the phylogeny of *Datura* plants, but they appear not to

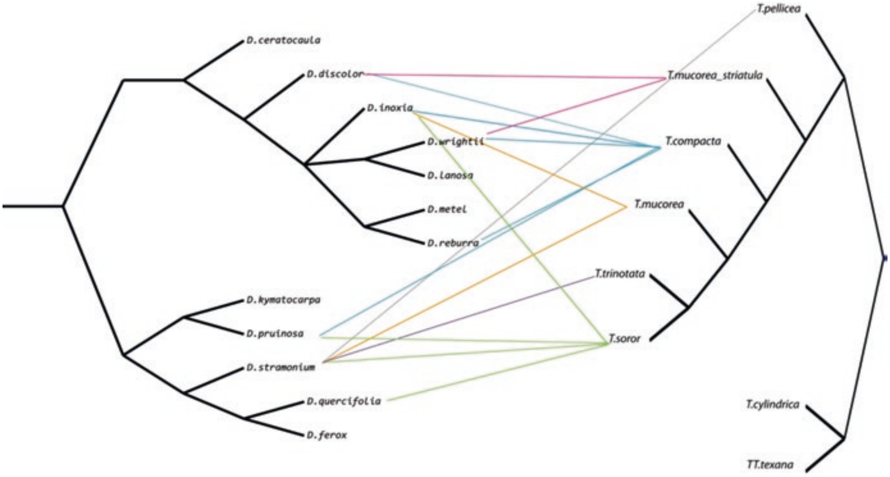

Fig. 14.1 Non-matching phylogenies of *Datura* (left) and *Trichobaris* (right) (phylogenies based on Kariñho-Betancourt et al. 2015 and De-la-Mora et al. 2018. Redraw with permission from authors)

be cospeciated (Fig. 14.1). Nonetheless, a more general trend was found in *Trichobaris* species; it becomes notorious that the sexual dimorphism of the weevils' rostrum might be associated with the plant tissue (presumably to dig dipper and oviposit the egg closer to the seeds; Fig. 14.2). The analyses of reconstruction of the ancestral host plant highlight the importance of the *Datura* plants for the diversification or *Trichobaris* species (Fig. 14.3). The most recent species uses the fruit of *Datura* plants in comparison with *T. texana* and *T. cylindrica* which use the stem of a *Solanum* plant.

The tracking of the host plant on haplotype COI networks of *Trichobaris* species (Fig. 14.4) shows low levels of specificity between weevils and their host. The most widespread species are *T. soror* and *T. compacta*. Even though both weevils did not show a clear lineages association to different plants, certainly *T. soror* looks more specialist than *T. compacta*, instead the widespread distribution both species. It is possible to visualize which host plant are the most common host for both species; *Datura stramonim* for *T. soror* and *D. wrightii* for *T. compacta.* 90% of the haplotypes in *T. soror* are distributed in *D. stramonium* and the remaining in *D. quercifolia*, *D. inoxia*, and *S. tuberosum*. In the case of *T. compacta*, it was found mainly in *D. wrightii* and also in *D. reburra*, *D. discolor*, and *D. pruinosa*.

As a summary, it is probable that the genus *Datura* has an influence in the evolution of *Trichobaris.* The ancestral plant could be a plant from the genus *Solanum*, and the ancestral *Trichobaris* should put their eggs in the stem of these plant. When the plants of the genus *Datura* originated, they start to use the fruits of these plants, as a consequence as the rostrum started elongated.

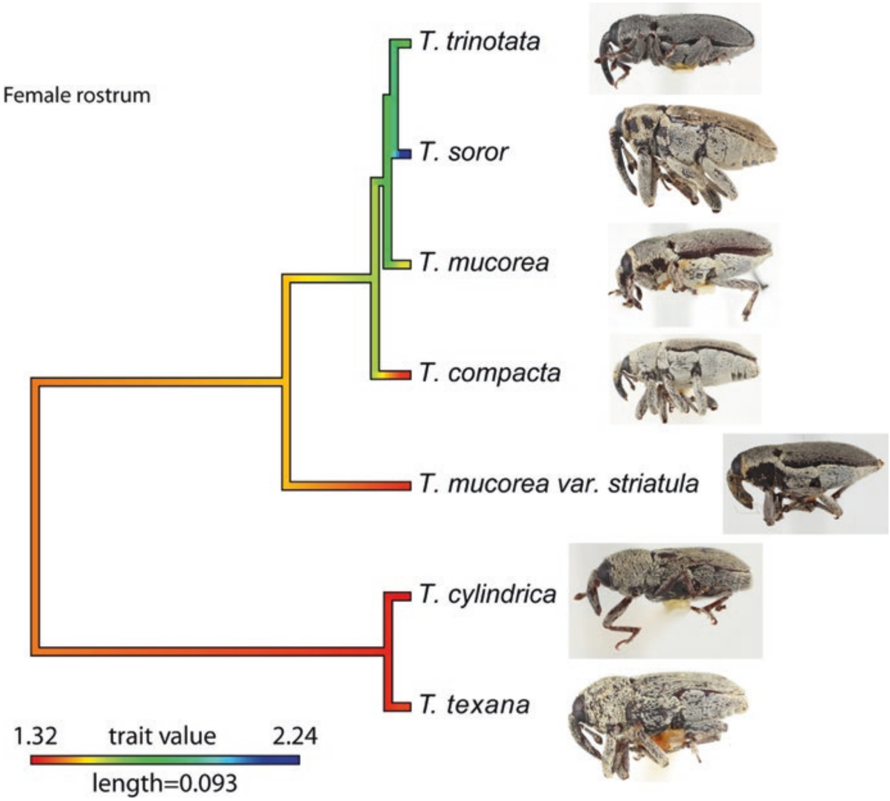

Fig. 14.2 Evolution of female rostrum of *Trichobaris* species. Pictures are not at scale (modified from De-la-Mora et al. 2018)

For the moment, this information makes us corroborate the general trends in the evolution of weevils associated with the elongation of the rostrum, the key role of the host plant as a selective pressure, not just by species if not because they offer several niches (as kind of tissues) that weevils can use as a resource and its promotes their genetic diversity.

Finally, the interaction among *Trichobaris* and their host plants is highly relevant for a biogeographic perspective for the assembly of interactions in the Mexican transition zone, an area where Neotropical and Nearctic biotas meet. These is because the origin of the Solanaceae has been identified in the Andean region of South America (Dupin 2017) and the origin of the genus *Datura* in the center of Mexico (Luna-Cavazos and Byes 2011), while the *Trichobaris* are of affinity Nearctic (Marvaldi et al. 2002). What make us think about the interactions themself promote biodiversity. To corroborate some biogeographic pattern, it would be good

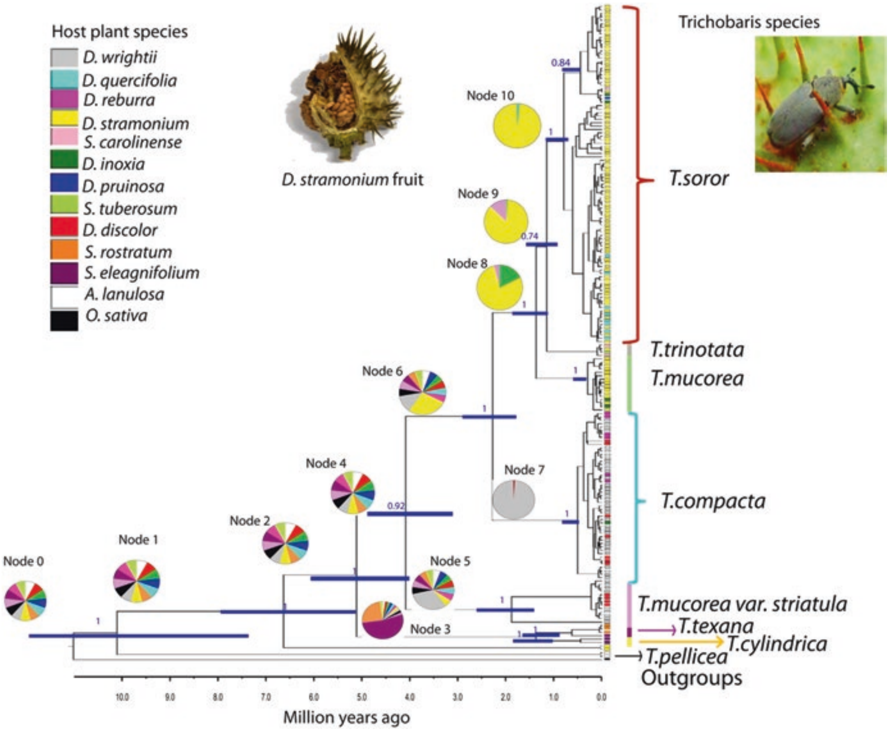

Fig. 14.3 Mapping the ancestral host plant on the *Trichobaris* COI haplotype phylogeny. Pie plots show the probability of a host plant species at each node in the phylogeny (modified from De-la-Mora et al. 2018)

to look other weevils and plants that present the same pattern of distribution, but again little is known about the host plant species.

As a conclusion, in the study of the diversity of the herbivorous insects, the plants are playing an important role but are not the unique source of selection driving divergence among insects. It is also important to considerate the trends in the evolution of the species or group that we are studying to discover general patterns and look closely to the interaction among insects and plants, considering all the geographical range of distribution of interacting species.

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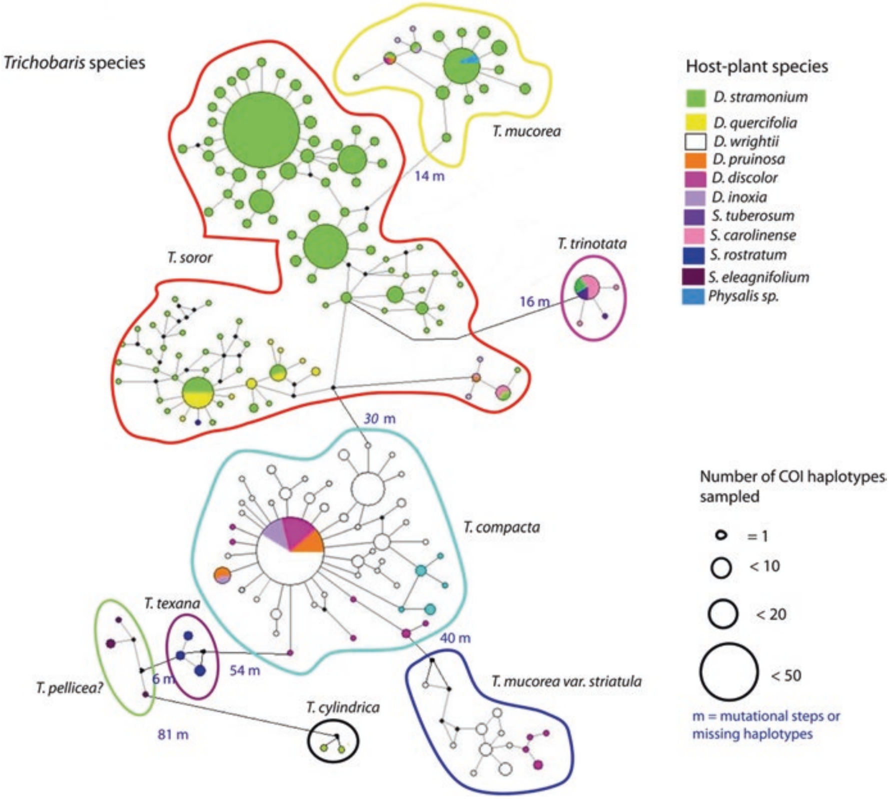

Fig. 14.4 Distribution of *Trichobaris* on host plant species across COI haplotype networks. Each haplotype is a circle, and its frequency is represented by the circle size. The color inside the circles shows the proportion of such haplotype sampled at a given host plant species. All haplotypes that correspond to the same *Trichobaris* species are border by the same color as indicated in the figure legend (modified from De-la-Mora et al. 2018)

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Chapter 15
The Evolution and Diversification of Neotropical Generalist Herbivores: The Evolutionary History of the Grasshopper Genus *Sphenarium* Charpentier, 1842

Salomón Sanabria-Urbán and Raúl Cueva del Castillo

Abstract We present the current understanding on the diversification of the Mesoamerican genus *Sphenarium,* a group of generalist-herbivorous grasshoppers that could play a major role on the evolution of defense mechanisms and life history traits of plants along to their distribution range. We discuss their phylogenetic relationships and how geological and climatic history, as well as environmental variation, could favor their expansion and diversification. Furthermore, in a phylogenetical framework, we considered future directions on the study of their interactions with the plants with which their populations have evolved.

Keywords Neotropical region · Diversification · Quaternary speciation · Cryptic diversity · Local adaptation

Introduction

The diversity of herbivores, their host plants, and the defensive adaptations of plants to herbivory are postulated to have arisen by a long history of coevolution that has affected the food web links between these trophic levels (Ehrlich and Raven 1964). The understanding of the macroevolutionary history of interactions requires the integration of ecology, evolution, and the role of history in shaping the diversification or decline of lineages (Futuyma and Agrawal 2009; Reznick and Ricklefs 2009). Phylogenies allow us to infer a major component of evolutionary history, namely, the sequence of divergence of lineages as portrayed in phylogenetic trees (Futuyma and Agrawal 2009). They play a fundamental role to understanding the

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dynamic of communities (Kraft et al. 2007; Buckley et al. 2010; Gerhold et al. 2015); the phylogenetic evidence allows us to examine the structure of community assemblages, exploring the basis of community niche structure and adding a community context to studies of trait evolution and biogeography (Webb et al. 2002; Cavender-Bares et al. 2012; Villalobos et al. 2016).

The Evolutionary History of the Genus *Sphenarium*

The Genus Sphenarium*, Its Higher-Level Phylogenetic Relationships, and Biogeographic Origin*

The genus *Sphenarium* Charpentier, 1842 comprises a monophyletic group of 17 fusiform flightless grasshopper species (Sanabria-Urbán et al. 2017). This genus belongs to the family Pyrgomorphidae (Orthoptera: Caelifera), an ancient lineage of grasshoppers of Gondwanan origin around 141 mya (Mariño-Pérez and Song 2019). This family comprises 482 extant species in 149 genera mainly distributed across the tropics of the world (Mariño-Pérez and Song 2018). However, most pyrgomorphs are found in the Old World (Africa, Asia and Australia), whereas only 41 species in 13 endemic genera are found in the New World, where *Sphenarium* is the most diverse genus (Cigliano et al. 2019).

Various phylogenetic and biogeographic hypotheses for the origin of the New World Pyrgomorphidae, including *Sphenarium*, have been proposed (Kevan 1977; Mariño-Pérez and Song 2019). However, only currently these hypotheses have been tested by Mariño-Pérez and Song (2019) using molecular information. They found that the New World Pyrgomorphidae consist of at least three separate clades spread throughout the phylogeny of the family. The first clade includes the genera in the South American endemic tribe Omurini and the genus *Jaragua* restricted to the Caribbean, the second clade comprises the genera in the Mexican endemic tribes Ichthiacridini and Ichthyotettigini, and the last clade includes the Mesoamerican genera *Sphenarium* and *Prosphena*. Interestingly, this last clade, *Sphenarium-Prosphena*, is more closely related to the African genera *Ochrophlegma* and *Tanita* than to other American pyrgomorphs. Moreover, these four genera (*Sphenarium-Prosphena-Ochrophlegma-Tanita*) are most closely related to the Mexican Ichthiacridini-Ichthyotettigini clade within the phylogeny of the family.

Given these phylogenetic relationships Mariño-Pérez and Song (2019) have inferred a complex biogeographic history for the New World Pyrgomorphidae that implicate at least two colonization events to America. The first and oldest event could involve vicariance or dispersal from Africa of the common ancestor of the South American and Caribbean lineage (Omurini-*Jaragua*) during the Cretaceous (112–81 mya). In both cases a subsequent dispersal event from South America to the Caribbean in the late Cretaceous (~68 mya) is also inferred. The second wave of colonization came by dispersal from West Africa to northern South America and then to North America and Africa, when the continents were already separated. The common ancestor that colonized South America for the second time in the late Cretaceous (~69 mya) gave rise over there to the clades *Sphenarium-Prosphena*, *Ochrophlegma-Tanita*, and Ichthiacridini-Ichthyotettigini. The common ancestors of these clades then dispersed to North America (*Sphenarium-Prosphena* and Ichthiacridini-Ichthyotettigini) and back to Africa (*Ochrophlegma-Tanita*), before the entire lineage became extinct in South America during the early Paleogene (60–50 mya). According with this scenario, the ancestors of *Sphenarium* could establish in North America since the Eocene (~ 50–40 mya), which may imply a relatively long history of evolution of *Sphenarium* grasshoppers in this region.

Ecology and Natural History of the Genus **Sphenarium**

The species of *Sphenarium* are distributed from central Mexico to northwestern Guatemala (Fig. 15.1), where the major mountain ranges delimit their parapatric distribution (Sanabria-Urbán et al. 2017). *Sphenarium* grasshoppers are found in a wide variety of plant communities, including xerophytic, temperate, tropical deciduous, and rain forests. These insects are mainly found in the border vegetation and in sunny areas, where they feed on weeds, shrubs, and even trees of a wide variety of seasonal and perennial plants species. At least 47 species in 25 families of vascular plants and ferns are known to be eaten by *S. purpurascens* (Table 15.1) (Cano-Santana and Castellanos-Vargas 2009; and references therein).

The diet breadth of the other species of *Sphenarium* has remained unstudied. However, these other species are commonly found in the same plant species and/or families in nature (Márquez 1962, 1965a, b; Descamps 1975; Oyama et al. 1994; Sanabria-Urbán, *pers. obs.*), suggesting that in general these insects are polyphagous herbivories.

The diet composition of the grasshoppers of *Sphenarium* could be determined by a mix of their food preferences and the encounter probability with their host plants. Moreover, the population densities of these insects can modulate their diet breadth. For instance, in low population densities, their diet breadth can be narrow, tending to feed mostly on soft tissue plants (e.g., seasonal Asteraceae), whereas when they reach high densities, their diet breadth can expand to include plants species with harder tissues, such as cactus (e.g., *Opuntia* sp.) (Cueva del Castillo, *pers. obs.*). Similar behaviors have been documented in other grasshoppers (Otte and Joern 1976; Bernays and Chapman 1977; Joern 1979), which can be explained by the quality and quantity of nutriments and the difficulty to obtain them from these types of plants.

Besides being polyphagous herbivorous, the species of *Sphenarium* can be very abundant and even show populations outbreaks (Kevan 1977). For instance, *S. purpurascens* can represent up to 95% of the dry biomass of the epiphytic arthropods in xerophytic habitats in central Mexico (Ríos-Casanova and Cano-Santana 1994). During their populations outbreaks, these insects can infest several crop plant

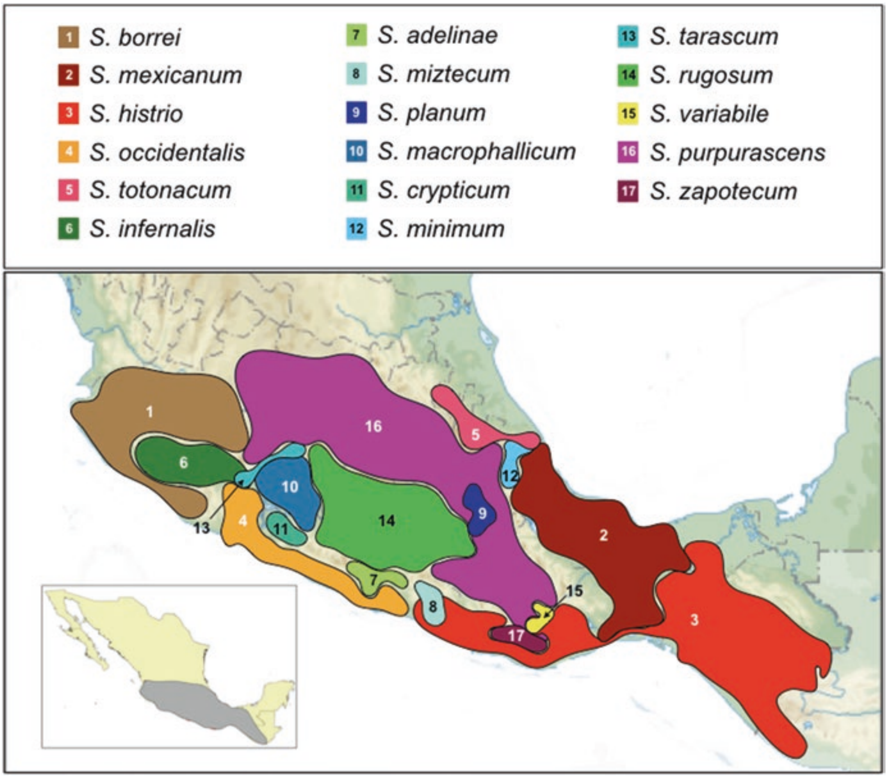

Fig. 15.1 Geographic distribution ranges of the species of *Sphenarium*. (After Sanabria-Urbán et al. 2017)

species. Indeed, *Sphenarium* grasshoppers have long been regarded as one of the most severe agricultural pests of corn and beans in central Mexico (Cerritos and Cano-Santana 2008). But at the same time, they have been used as food since pre-Colombian times for Mexican people (Ramos-Elorduy and Moreno 1989). Other crop pest species are also recognized in at least ten genera of Pyrgomorphidae, but all of them in the Old World, mainly in Africa and Asia (Table 15.2).

Even though studies on the phenology of *Sphenarium* grasshoppers have focused mainly in one species, *S. purpurascens* (Cano-Santana and Castellanos-Vargas 2009; and the references therein), several lines of evidence suggest that all species in the genus are phenologically similar (Márquez 1962, 1965a, b; Descamps 1975; Sanabria-Urbán et al. 2015, 2017). These grasshoppers are univoltine. Their nymphs emerge mainly in the beginning of the rainy season (June–July), and they become adults and reproduce mainly during the fall (from mid-September to mid-December). After reproduction the oviposition and the highest adult mortality occur during the winter (approximately from mid-December to mid-February) (Sanabria-Urbán et al. 2015, 2017). However, the species of *Sphenarium* show extensive variation at inter- and intraspecific levels on body size and life history traits (Kevan 1977;

Table 15.1 Plant families and species known to be eaten by *Sphenarium purpurascens*

Family	Plant species	Family	Plant species
Amaranthaceae	*Amaranthus* sp.	Hydrophyllaceae	*Wigandia urens*
	Iresine calea	Hypoxidaceae	*Hypoxis decumberus*
	Iresine celosa	Lamiaceae	*Salvia mexicana*
Anacardiaceae	*Schinus molle*	Leguminosae	*Cologania* sp.
Asparagaceae	*Manfreda brachystachya*	Loasaceae	*Eysenhardtia polystachya*
Begoniaceae	*Begonia gracilis*		*Gliricidia sepium*
Burceraceae	*Bursera* sp.		*Phaseolus heterophyllus*
Commelinaceae	*Tradescantia crassifolia*		*Mentzelia hispida*
Compositae	*Baccharis ramulosa*	Loganiaceae	*Buddleja cortada*
	Dahlia coccinea		*Buddleja parviflora*
	Eupatorium petiolare	Nyctaginaceae	*Mirabilis jalapa*
	Gnaphalium canescens	Plantaginaceae	*Penstemon campanulatus*
	Helianthus annuus	Poaceae	*Plantago major*
	Lagascea rigida		*Melinis repens*
	Montanoa tomentosa	Poaceae	*Tripsacum lanceolatum*
	Piqueria trinervia	Polygonacea	*Rumex obtusifolius*
	Senecio praecox	Polypodiacea	*Polypodium* sp.
	Stevia ovata	Rubiacea	*Bouvardia ternifolia*
	Stevia salicifolia	Sapindaceae	*Cardiospermum halicacabum*
	Tagetes micrantha	Solanaceae	*Datura stramonium*
	Tithonia sp.		*Nicotiana glauca*
	Verbesina virgata		*Physalis* sp.
Crassulaceae	*Echeveria gibbiflora*		*Solanum* sp.
Dioscoreaceae	*Dioscorea galeottiana*	Vitaceae	*Cissus sicyoides*
Euphorbiaceae	*Acalypha indica*		

Sanabria-Urbán et al. 2015, 2017). These traits are common targets of natural selection, and their geographic variation suggest high levels of adaptation to environmental heterogeneity across their distribution (Sanabria-Urbán et al. 2015). Moreover, morphological and behavioral traits appear to be under strong sexual selection (Cueva del Castillo and Nunez-Farfan 1999, 2002; Cueva del Castillo et al. 1999). For instance, in *S. purpurascens*, larger males have advantage in accessing females (Cueva del Castillo et al. 1999) and show prolonged female guarding behavior (spending up to 22 days mounted on females) that may suggest strong sperm competition (Cueva del Castillo 2003).

Most *Sphenarium* species exhibit apparently cryptic coloration patterns, but in some species (e.g., *S. purpurascens*, *S. histrio*, *S. mexicanum*, and *S. mixtecum*) brightly colorations are relatively common (Sanabria-Urbán et al. 2017), resembling aposematic pyrgomorphs from the Old World (Mariño-Pérez and Song 2018). Aposematism and the ability to sequester secondary compounds from toxic plant have been documented in about 10% of the species of Pyrgomorphidae (Mariño-Pérez and Song 2018). However, it seems that these traits have not evolved

Table 15.2 Grasshopper species of the family Pyrgomorphidae from Africa and Asia that have been recognized as crop pest

Species	Region	Country	Attacked crops	References
Atractomorpha burri	Asia	India, Filipinas	Rise	Ane and Hussain (2015)
Atractomorpha crenaticeps	Asia	Indonesia (Java)	Sugarcane	Jarvis (1927)
Atractomorpha crenulata	Asia	India	Cotton, sugarcane, cauliflower, chickpea, paddy, maize, milch, millet. jute, gram. oat, cow pea, tobacco, oriental pickling melon	Jago (1998), Thakur and Thakur (2011), Gupta and Chandra (2013), Patra et al. (2013), and Debbarma et al. (2017)
Atractomorpha lata	Africa, Asia	Cameroon, Japan, Korea	Rice, and it is also pest to over 132 medicinal plant species	Kobayashi et al. (1972), Lee et al. (2007), and Seino and Njoya (2018)
Atractomorpha sinensis	Asia	China, Taiwan, Mongolia, Vietnam	Ornamental plants, rice paddy, cotton, sugarcane, pineapple	Kevan and Hsiung (1985)
Aularches miliaris	Asia	India	Jack fruit, coconut, *Pinus*, *Shorea robusta*, coppica shoots, banana, beans, betel nut, cocoa, cardamom, cashew, chili, *Cinchona*, cotton, millet, guava, jute, maize, mango, rice, rubber, sugarcane, tobacco	Gupta and Chandra (2013)
Chrotogonus oxypterus	Asia	India	Cotton, sorghum, maize, wheat, groundnut	Tandon (1986) and Gupta and Chandra (2013)
Chrotogonus trachypterus	Asia	India Filipinas	Rise, cauliflower, minor pests of cotton, sorghum, maize, wheat, groundnut, tobacco, and paddy	Mahabir (1980), Thakur and Thakur (2011), and Ane and Hussain (2015)
Colemania sphenaroides	Asia	India	Jowar, bajra, sugarcane, and millets	Gupta and Chandra (2013)
Hieroglyphus banian	Asia	India, Pakistan	Rice, maize, cotton	Gupta and Chandra (2013)
Neorthacris acuticeps	Asia	India	Minor damage the tea plantation; low growing crops	Gupta and Chandra (2013)
Neorthacris simulans	Asia	India	*Rauwolfia serpentina*	Gupta and Chandra (2013)
Neorthucrts acuticeps	Asia	India	*Costus speciosus*	Swamy et al. (1993)

(continued)

Table 15.2 (continued)

Species	Region	Country	Attacked crops	References
Poekilocerus pictus	Asia	India	Alfalfa, crop, citrus, melon, papaya, chili, cucurbit, orchards, betel, creepers, forest trees, trees of jasmine, mulberry	Soomro et al. (2014)
Pyrgomorpha granulata	Africa		It sometimes attacks cotton and other crops	Ferdio and Cardoso (1972); Mason (1979), Kevan and Chia-Chi Hsiung (1985)
Pyrgomorpha vignaudi	Africa	Nigeria	Cowpea, soya bean, rice, *Ceratotheca sesamoides*, millet, amaranth, green gram	IITA (1984), Fassakin (1991), Heinrichs and Barrion (2004), Adamu et al. (1999), Paraïso et al. (2012), and Kekeunou et al. (2006)
Taphronota thaelephora	Africa	Cameron	Coffee	Seino et al. (2013)
Zonocerus elegans	Africa	Kenia	Sunflower	Khaemba and Mutinga (1982)
Zonocerus variegatus	Africa	Nigeria	Cassava, coffee	Page et al. (1980) and Jago (1998)

in the group. For instance, morphological and molecular specializations related with aposematisms in pyrgomorphs have not been observed in *Sphenarium* grasshoppers (Mariño-Pérez and Song 2018; Yang et al. 2019). In fact, these grasshoppers are heavily predated by multiple species of arthropods, lizards, birds, and mammals, including humans (Cano-Santana and Castellanos-Vargas 2009; Sanabria-Urbán, *pers. obs.*). Thus, it seems unlikely that these grasshoppers are toxic for their predators. Nevertheless, some species in the genus (e.g., *S. purpurascens* and *S. rugosum*) can feed on toxic plants, such as *Datura stramonium* (Castillo et al. 2014; Sanabria-Urbán, *pers. obs.*), and generate enormous damage to them (Núñez-Farfán and Dirzo 1994; Fornoni et al. 2003; Castillo et al. 2014), despite of *D. stramonium* has well-known defense mechanisms against herbivores (e.g., trophane alkaloids and trichomes) (Valverde et al. 2001). So far, it remains largely unknown what are the mechanisms that have allowed *Sphenarium* species to feed on their multiple host plant species.

The Phylogenetic Relationships Among the Species of Sphenarium

The most comprehensive phylogenetic study on *Sphenarium* until now was conducted by Sanabria-Urban et al. (2017). They found that after the divergence from its sister genus *Prosphena*, the common ancestor of *Sphenarium* gave raise to three major clades that diversified subsequently (Fig. 15.2). These clades are geographically restricted to particular regions across the distribution of the genus. The first and most basal clade is just composed by the species *S. borrei*, which is restricted to the inner highlands of western-central Mexico. The second clade is composed by the species *S. totonacum*, *S. occidentalis*, *S. mexicanum*, and *S. histrio* that are distributed in the costal lowlands and the highlands of southern Mexico and

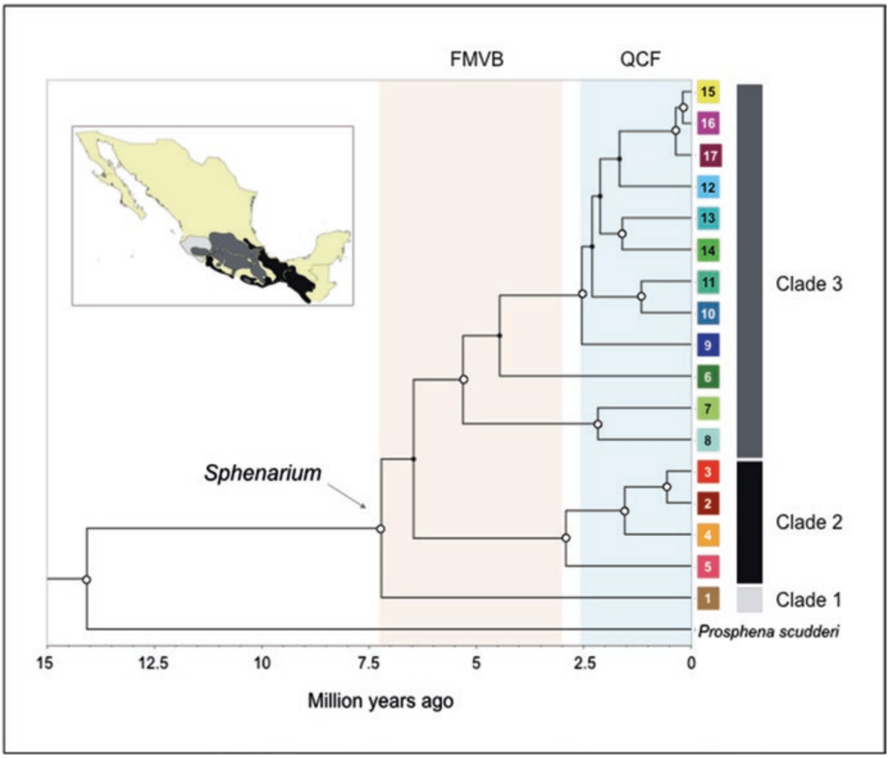

Fig. 15.2 Phylogenetic relationships and mean divergence times between the species of *Sphenarium* based on a Bayesian species tree analysis of the genus (after Sanabria-Urban et al. 2017). The geographic distribution and species within the three major clades of *Sphenarium* are represented by the gray shapes and rectangles. Each species is represented by different colored squares (same as in Fig. 15.1). Nodes with posterior probability values ≥0.8 are indicated with open circles. The temporal occurrence of the second major formation of the Mexican Volcanic Belt (FMVB) and Quaternary climatic fluctuations (QCF) is denoted by the light-colored areas on the chronogram

northwestern Guatemala. The third clade comprises all the species distributed in the inner basins and highlands of central Mexico: S. *infernalis, S. adelinae*, *S. mixtecum*, *S. planum*, *S. minimum*, *S. macrophallicum*, *S. crypticum*, *S. rugosum*, *S. tarascum*, *S. zapotecum*, *S. variabile*, and *S. purpurascens* (Fig. 15.2). A closer phylogenetic relationship is recovered between the last two clades but with poor support.

The species within the last two clades integrate into different nested monophyletic groups. In the second clade, the basal position is occupied by *S. totonacum*, followed by *S. occidentalis*, which is closely related to *S. mexicanum* and *S. histrio*. In the third clade, sister relationships were recovered between *S. adelinae* and *S. mixtecum*, *S. crypticum* and *S. macrophallicum*, and *S. rugosum* and *S. tarascum*, and between *S. purpurascens*, *S. variabile*, and *S. zapotecum*. The last seven species conform a monophyletic group along with *S. minimum* and *S. planum*. Nonetheless, the phylogenetic relationships between these groups of species and the other species in the clade are poorly supported. The basal positions in this last monophyletic group are occupied by the species in the western range of the clade (*S. adelinae*, *S. mixtecum*, and *S. infernalis*), followed by the species distributed in the Tehuacan Valley (*S. planum*), the Balsas River Basin (*S. crypticum*, *S. macrophallicum*, *S. rugosum*, and *S. tarascum*), and in the southern Sierra Madre Oriental (*S. minimum*), whereas the most recently derived species are those distributed in the highlands of central and southern Mexico (*S. purpurascens*, *S. variabile*, and *S. zapotecum*) (Fig. 15.2). The low phylogenetic resolution of some of the basal divergences, as well as the fact that some morphologically different sister species were found to be paraphyletic in their genetic lineages (*S. histrio*-*S. mexica*num; *S. macrophallicum*-*S. crypticum*; *S. rugosum*-*S. tarascum*; and *S. purpurparcens*-*S. variabile*-*S. zapotecum*), can be explained by incomplete lineage sorting associated with relatively rapid and/or simultaneous cladogenetic events.

Historical Biogeography of the Genus

According with Sanabria-Urbán et al. (2017), the initial divergences in *Sphenarium* (between the clades and the basal lineages within them) occurred between 2.91 and 7.22 mya, whereas most of the cladogenetic events within the clades occurred between 0.19 and 2.51 mya (Fig. 15.2). These two major episodes of diversification correlate temporally and spatially with the third major formation of the Mexican Volcanic Belt (MVB), around 3–7.5 mya (Ferrari et al. 2012), and the Quaternary climatic fluctuations around 0.01–2.6 mya. These historic events are recognized as some of the most important drivers of lineage diversification in other co-distributed taxa in Mexico (Bryson et al. 2011, 2012; Duennes et al. 2012; Mastretta-Yanes et al. 2015).

The several volcanic episodes during the formation of the MVB (Ferrari et al. 2012) probably sundered ancestral populations of *Sphenarium*, causing to the divergences between the three major clades, whereas Quaternary climatic changes probably caused several vicariance events within the clades by promoting recurrent distribution shifts of the ancestral populations across the mountain ranges and the costal lowlands of Mexico. In addition, the fact that strongly supported monophyletic groups of *Sphenarium* are geographically restricted to well-defined biogeographic provinces (Figs. 15.1 and 15.2) indicates that vicariance events could have played a fundamental role on the diversification of the group. Therefore, the current parapatric distribution of the species, along with their narrow sympatric zones, has probably resulted from secondary dispersal events. Moreover, the phylogenetic relationships among the lineages of *Sphenarium* suggest that probably the common ancestor of the group occupied initially the outer lowlands and that younger lineages have more recently colonized inner basins and highlands of central Mexico.

The Mechanisms of Differentiation Among the Species

The genus *Sphenarium* is an assemblage of lineages with different levels of morphological and genetic divergence suggesting a complex interplay between evolutionary forces during the evolution of the genus. There are three broad patterns of differentiation that reflect the relative importance of evolutionary forces on the diversification of *Sphenarium* (Sanabria-Urbán et al. 2017). Firstly, despite some species pairs are very close genetically (e.g., *S. histrio*-*S. mexicanum*; *S. macrophallicum*-*S. crypticum*; *S. rugosum*-*S. tarascum*; and *S. purpurascens*-*S. variabile*), they strongly differ from each other by male genital morphology. Because male genitalia are known to be under strong sexual selection (Eberhard 2010), and sexually selected characters tend to diverge very rapidly (Hosken and Stockley 2004), sexual selection may have played a major role in the divergence among these *Sphenarium* species. A second pattern is found among species that are morphologically similar (e.g., *S. miztecum*-*S. adelinae*; *S. histrio*-*S. occidentalis*; and S. *infernalis*-*S. rugosum*) but strongly differ genetically, reveling cryptic diversity in the genus. In these cases, evolutionary processes different from sexual selection on male genitalia could have played an important role in the divergence of lineages. Recurrent isolation events and genetic drift in ancestral populations could have generated genetic rather than morphological divergence. The last pattern involves species that differ both morphologically and genetically (e.g., *S. borrei* and *S. totonacum*), which suggest an interplay between different evolutionary forces (e.g., drift and sexual selection) in driving the species differentiation. In addition, Sanabria-Urbán et al. (2015) found correlative evidence suggesting that natural selection on body size in response to altitudinal climatic variation could have also promoted the diversification of the genus *Sphenarium*. Despite phylogenetic relationships have heavily affected the body size and the climatic niche of *Sphenarium* species, they have diverged considerably in size, and large species are associated with high temperatures during the winter (Sanabria-Urbán et al. 2015). This climatic body size cline probably reflects the life history adaptability of *Sphenarium* grasshoppers. In lowlands, during benign winters, the window for development and reproduction may increase, allowing grasshoppers to achieve larger body sizes. Conversely, when mean temperatures are lower, body sizes become smaller. Similar body size clines associated with decreasing temperatures have been observed in other insects at higher latitudes (Roff 1980; Dingle et al. 1990; Berner et al. 2004). Smaller body sizes at low temperatures are commonly explained by natural selection favoring faster development by decreasing development time (reducing the number of nymphal instars or diapause (Dingle et al. 1990) or increasing growth rates (Hodkinson 2005). However, decreasing the time to maturity at low temperatures may have negative effects on the fitness of individuals by reducing reproductive success via small body sizes (Mousseau and Roff 1987; Abrams et al. 1996; Morbey 2013). Therefore, the smallest species of *Sphenarium* (*S. purpurascens*, *S. minimum*, *S. planum*, *S. variabile*, and *S. tarascum*) probably have lower fecundity than larger species, but they have been able to colonize highlands.

The geologic and climatic events over the last 10 mya in Mexico had a profound impact on the diversification of the genus *Sphenarium* causing the vicariance of the ancestral lineages within this genus along their distribution. These historic events also determinate in great extent the complex mosaic of environmental heterogeneity to which *Sphenarium* species have adapted. The low mobility of these univoltine and flightless grasshoppers, plus the combination of strong natural selection on adult body size and maturation times, could enhance the genetic isolation and consequently the speciation of these Neotropical grasshoppers.

Perspectives

The current understanding of the evolutionary history of *Sphenarium* grasshoppers provides the opportunity to investigate many aspects of the interaction between these grasshoppers and their host plant species. Given that the species of *Sphenarium* are polyphagous insects that can reach high population densities; they can represent a strong selective force for several plant species across their distribution range. However, the strength of their selective pressure on their host plants might differ geographically and temporally depending on both, the variation in their own populations' densities and the variation on the composition and abundance of the plant in their communities. In those areas where these grasshoppers are more abundant, they might impose a stronger selective pressure on the plants. On the other hand, the distribution and abundances of their different host plant species also vary geographically and temporally. Thus, for some plant species and in some of their populations, these insects can be a more important selective factor than in others. Even though *Sphenarium* are generalist herbivorous, perhaps they have evolved in a complex mosaic of ecological interactions, generating places with high and low levels of coadaptation between them and their host plants. A recent study has depicted part of this complexity detecting geographic variation in defensive characteristics of the toxic plant *D. stramonium* associated with the abundance of *S. purpurascens* (Castillo et al. 2014). In some areas this grasshopper species exerts a strong selective pressure in *D. stramonium* toward reduction of the alkaloid atropine, whereas in other populations, a more derived alkaloid confers a greater defense. These results are congruent with geographic mosaic of adaptation in *D. stramonium,* in which *Sphenarium* grasshoppers are involved. Similar studies in other plant species could reveal similar responses, adding to our understanding of the relative importance that these grasshoppers have had on the evolution of defense mechanisms in plants. In this context, it should be considered the biogeographic history of the genus *Sphenarium*, and how it has influenced the geographic distribution of its host plant species. The distribution of *Sphenarium* species can generate geographic variation on the levels of herbivory which could explain the geographic distribution of some plant species. For instance, the sunflower (*Helianthus annuus*) reaches its southern distribution limit in the Mexican tropics, where *Sphenarium* species and other herbivores exert a tremendous herbivory pressure on the species (Lentz et al. 2008). The plants' ability to colonize a new area can be constrained by its native generalist herbivores because the foreign plants may be poorly adapted to defend themselves against them (Avanesyan and Culley 2015). However, coadaptation to these herbivores may eventually happen allowing the foreign plants to expand their distribution ranges in the new areas (Schaffner et al. 2011). Therefore, dominant generalist herbivores, such as *Sphenarium* grasshoppers, might restrict more strongly the distribution of recently established plant linages, in comparison with older clades that have coexisted with these insects during their evolution. These predictions can be tested by obtaining phylogenetic and biogeographic reconstructions of the different host plant lineages of *Sphenarium*. On the other hand, phylogenetic and biogeographic reconstructions of both, *Sphenarium* grasshoppers and their host plants, would allow to infer how old the interactions between *Sphenarium* species and their host plants are. In addition, the phylogenetic information would provide the bases to search for common adaptations among different host plants on defense and/or tolerance against these generalist grasshoppers. Finally, this would help to better understand how the evolutionary history of *Sphenarium* has been influenced by the diversification of their host plants and vice versa.

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Part IV
Genomics

Chapter 16

What Do We Know About the Genetic Basis of Plant Defensive Responses to Herbivores? A Minireview

Ivan Mijail De-la-Cruz, Sabina Velázquez-Márquez, and Juan Núñez-Farfán

Abstract Plants are frequently attacked by herbivores and pathogens and have evolved constitutive and induced defences to prevent/diminish fitness costs. Here, we review recent progress in the study of the defence genes in plants. The sophisticated signalling network of plant defence responses is elicited and driven by both herbivore-induced factors (e.g. elicitors, effectors, and wounding) and plant signalling (e.g. phytohormone and plant volatiles) in response to arthropod factors. Genome-wide data offer many advantages over sparser sets of genetic markers. It is now possible to detect selection across the genome and detect if those selected genes are associated with the herbivory. Genomic tools are now allowing genome-wide studies, and recent theoretical advances can help to design research strategies that combine genomics and field experiments to examine the genetics of local adaptation (cf. Savolainen et al. 2013). Plant and arthropod genomics provide many opportunities to understand the plant immunity to arthropod herbivores. Also, it will provide new insights into basic mechanisms of chemical communication and plant-animal coevolution and may also facilitate new approaches to crop protection and improvement.

Keywords Crosstalk · Herbivory · Local adaptation · Phytohormonal · Plant defences · Quantitative trait loci

The evolutionary history of terrestrial plants and their arthropod associates are inextricably linked (Smith and Clement 2012) since plants are the food source of nearly one million or more insect species from diverse taxonomic groups (Howe and

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Jander 2008). Because plants are sessile organisms and have no chance to escape from the attack of herbivores, they evolved particular strategies to defend themselves (Mithöfer and Boland 2012). Likewise, phytophagous arthropods have evolved ways to overcome plant defence (Futuyma and Agrawal 2009). This coevolutionary relationship is based on an inherent feature of life on Earth in which land plants and herbivores have continually adapted to changing environments and biotic pressures to survive (Futuyma and Agrawal 2009).

Plant traits that confer resistance to insect pests can be classified according to the manner in which they are deployed (Howe and Jander 2008). Some traits are expressed constitutively under the control of hard-wired developmental programmes, irrespective of the herbivore threat level, and if they are present or not (Howe and Jander 2008) (Figs. 16.1 and 16.2). In contrast, other plants defences, such as toxins, are induced, and other defences are only expressed in response to herbivory and at the site of tissue damage. In many cases, defences are produced systemically in undamaged tissues (Bostock 2005) (Figs. 16.1 and 16.2). Plants' induce defences are a sort of "elegant" defence system that can recognize the non-self-molecules or signals from damaged cells, much like the animals, activating the plant's immune response against herbivores (Hare 2010; Howe and Jander 2008; Verhage et al. 2010). Induced defence is mediated, in general, by the recognition of specific cues, for instance, herbivore-associated molecular patterns (HAMPs) in their oral secretions, followed by the elicitation of complex signalling networks, involving mitogen-activated protein kinase (MAPK) cascades as well as signalling via the Jasmonic acid, salicylic acid (JA, SA), and ethylene pathways (Campos et al. 2014). This signalling, in turn, leads to a reconfiguration of the transcriptome and proteome, as well as the biosynthesis of defence chemicals (Wu and Baldwin 2010) (Fig. 16.2). Induced and constitutive defence can be displayed directly as physical barriers (tissue toughness, plant pubescence, and glandular and nonglandular trichomes) or allelochemicals in plant tissues exhibiting antifeedant, toxic, or repellent effects on herbivores (Mithöfer and Boland 2012) (Fig. 16.1).

Defences also can be displayed indirectly as volatile organic compounds (VOCs) and green leaf volatiles (GLVs) (Fig. 16.1). These compounds are released by herbivore-damaged plants that attract arthropod predators and parasitoids or that may repel oviposition of pest arthropods (Baldwin et al. 2006; Kessler and Baldwin 2001, 2002). For example, HAMPs are specific plant indirect defence responses to specific herbivore-derived elicitors in oral or ovipositor secretions that facilitate indirect defences against herbivores (Mithöfer and Boland 2012) (Fig. 16.1). The most-studied HAMPs are insect fatty acid-plant amino acid conjugates from lepidoptera larvae (Halitschke et al. 2001; Kessler and Baldwin 2002; Schmelz et al. 2012).

In summary, constitutive and induced defence are regulated by complex physiological, biochemical, and molecular processes such as chemical signal cascades involving jasmonic acid (JA), salicylic acid (SA), ethylene, abscisic acid, and gibberellic acid that result in a downstream production of direct and indirect defences (Smith and Clement 2012) (Fig. 16.2). Despite the wealth of information on plant defence to natural enemies, our understanding of plants communication with

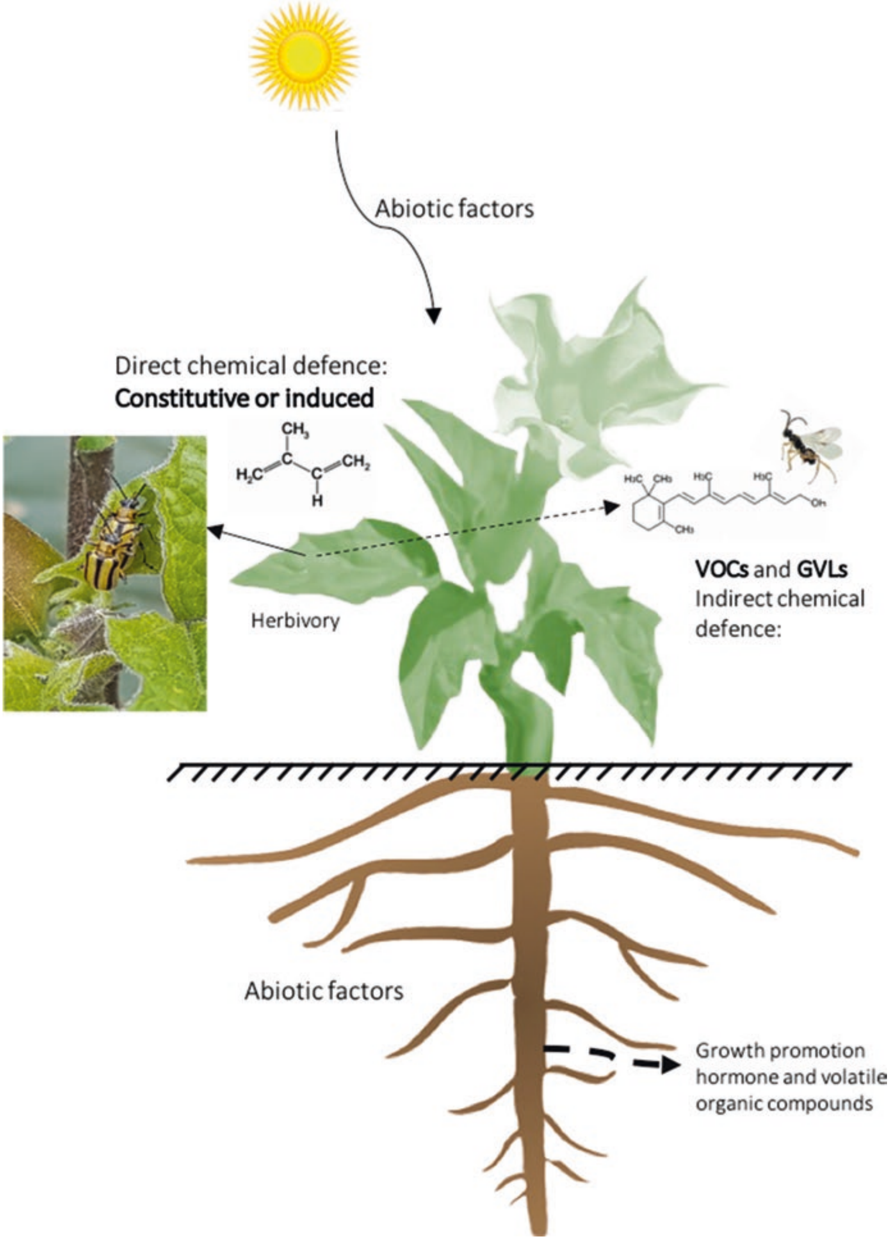

Fig. 16.1 Plants are subject to different environmental pressures from biotic or abiotic factors. In particular, plants interact with different organisms below- and aboveground. Belowground interactions include associations with mycorrhizal fungi, bacteria, and worms that feed upon the roots. Above the ground, insects feed on different plant tissues. Therefore, plants must deploy different defence strategies. These can be direct or indirect. Direct defences involve the production of different chemical weapons that can be constitutive or induced. Likewise, indirect defences include the release of VOCs and GLVs that attract predators of insects that feed on plants. Likewise, root exudates have been reported as defence against nematodes and pathogens. These exudates may have a role as an "attractant" to beneficial organism such as mycorrhizal fungi and bacteria. *VOC*s volatile organic compounds, *GLV*s green leaf volatiles

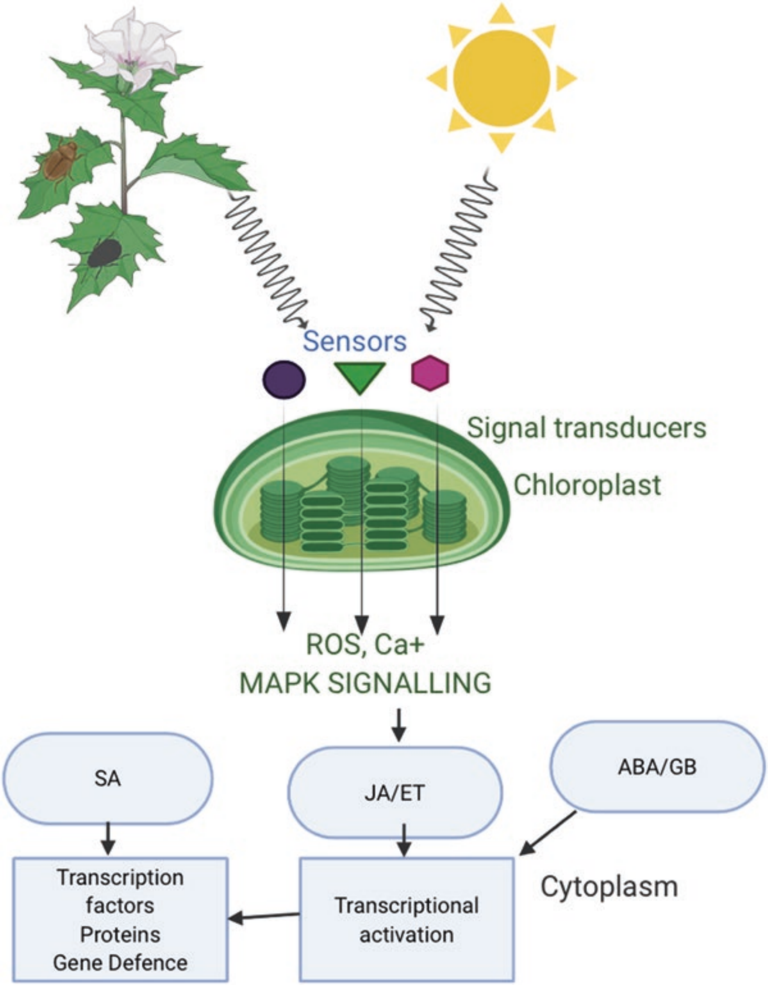

Fig. 16.2 Figure based on the model proposed by Howe and Jander (2008) that summarizes the regulation of jasmonate-based defences in response to herbivory. Biotic factors (herbivory) and abiotic factors such as red-light assimilation (black and red arrows) activate MAP kinases and the production of jasmonates such as jasmonoyl-isoleucine (JA-Ile). JA-Ile promotes SCFCOI1-mediated degradation of jasmonate ZIM domain (JAZ) repressor proteins, resulting in derepression of transcriptional regulons that control direct and indirect defensive traits. The jasmonate signalling pathway also regulates plant responses to developmental cues and other stress conditions

neighbours, symbionts, pathogens, herbivores, and herbivores' natural enemies, above- and belowground, is still limited (War et al. 2012). Much of the understanding of the molecular mechanisms and evolutionary origins of immune recognition in plants derives mainly from studies of plant-pathogen interactions (Howe and Jander 2008; Jones and Dangl 2006) and several other studies of plant-herbivore interactions in model plants (Howe and Jander 2008). In particular, there is little information about the genetic basis of plant defence traits for insects' herbivores. In this brief review, we focus on some fascinating examples of the genetic basis of defence in plants. We highlight the recent advances in the understanding of some defence genes and (1) discuss how herbivores can drive the genetic evolution of defence traits in plants; (2) we emphasize the different gene responses to a combination of stressors, such as drought + herbivory, and the genetic architecture of defence traits (i.e. whether few or many genes do explain a high fraction of phenotypic variance in plant defence). (3) We also discuss on the role of elicitors in the plant-herbivore interaction, (4) and on the role of the phytohormones, transcriptions factors, and epigenetic mechanisms that regulate the defence genetic responses. (5) Finally, we point out some future directions in the study of plant-herbivore interactions in the light of genomics.

Herbivores as Selection Agents on Defensive Genes

Geographic analyses of genetic variation in several plant species indicate clear genetic signals of local adaptation (Linhart and Grant 1996) caused by spatial differences in selection (Johnson et al. 2018; Züst et al. 2012). Insects use different feeding strategies to obtain nutrients from all above- and belowground plant parts (Howe and Jander 2008). Although all phytophagous insects inflict mechanical damage on plant tissues, the quantity and quality of injury vary greatly, depending on the feeding tactic (Howe and Jander 2008). Although evidence indicate that climate and soil variability can exert strong local selective pressures and play essential roles in shaping large-scale plant genetic patterns (Hancock et al. 2011), there is less direct evidence that biotic forces, such as herbivory or competition, can lead to the maintenance of genetic variation across broad geographic scales (Howe and Jander 2008). For instance, quantitative analyses show that *GS-ELONG* locus constitutes an insect resistance QTL, caused by variation in glucosinolate quantity, quality, or both in *Arabidopsis thaliana* (Kroymann et al. 2003). Züst et al. (2012) also studied alleles of the *GS-ELONG* and *GS-AOP* loci that mechanistically determine the accumulation and structure of aliphatic glucosinolates. Aliphatic glucosinolates are the first defence in *Arabidopsis thaliana,* and the *GS-ELONG* locus regulates the carbon side-chain elongation (*3C* or *4C*) (Kroymann et al. 2003), whereas the *GS-AOP* locus modifies the functional group of the biologically active glucosinolate side chain (*ALK*, *OH*, or *NULL*). The combination of these alleles yields six distinct chemotypes present in natural populations in varying proportions (Chan et al. 2011; Züst et al. 2012). The authors mapped the geographic variation in the abundance of the six chemotypes within Europe, from a set of 96 accessions with known chemical profiles, and found that the frequency of *3C* to *4C* chemotypes of *GS-ELONG* locus increased with latitude and longitude and that the herbivores *Brevicoryne brassicae* and *Lipaphis erysimi* drive these chemical patterns as both are abundant.

In addition, a multigenerational selection experiment with populations of *A. thaliana* attempted to measure aphid selection on *GS-ELONG* (Züst et al. 2012). Over 5 generations they exposed 30 populations to replicated ($n = 6$) treatments of a single specialist aphid species (*B. brassicae* or *L. erysimi*), a single generalist aphid (*Myzus persicae*), a mixture of the three aphid species, and a treatment with no aphids. Züst et al. (2012) detected rapid adaptation in the selection experiment as evidenced by a progressive reduction in the effect of aphids' feeding on final plant biomass in each generation: *L. erysimi* caused the strongest reduction in plant biomass, *M. persicae* had an intermediate effect, and *B. brassicae* had the least effect. The mixture treatment caused a reduction similar to that produced by *L. erysimi* alone. Also, trichome density decreased in the treatment with no aphids each generation but remained high in all aphid treatments. Adaptation to herbivore feeding produced considerable changes in the genotypic composition of populations, including the complete loss of nine genotypes (Züst et al. 2012). In contrast, the different aphid treatments had a marked effect on the dominant aliphatic chemotypes within experimental populations. Markedly, the relative proportions of *3C* and *4C* chemotypes differed strongly among aphid treatments. After selection, populations with no aphids consisted of approximately two-thirds *3C* and one-third *4C* chemotypes. Therefore, specialist aphids selected for different chemotypes at *GS- ELONG* are as follows: the *4C* chemotypes strongly dominated in *B. brassicae* treatments and the *3C* chemotypes strongly dominated in both *L. erysimi* and the aphid mixture treatments. The genotypic composition of plant populations with *L. erysimi* and aphid mixtures was almost identical, confirming that *L. erysimi* dominated the mixture treatments and suggest that in co-founded populations, *L. erysimi* is the most important selective agent in populations of *A. thaliana* in Europe. Most successful genotypes had either a *3C-OH* or a *4C-NULL* chemotype, and no individuals belonging to either alkenyl chemotype (*3C-ALK* or *4C-ALK*) were found in any treatment. This study is strong evidence that the magnitude and direction of selection exerted by two specialist aphids on *GS-ELONG* drives the changes in the chemistry of *Arabidopsis* across Europe.

Rausher and Huang (2016) investigated how long individual plant defensive genes are involved in the coevolutionary process. They assessed the patterns of selection on the defensive gene threonine deaminase (*TD*) which is found in several Solanaceae species as *Solanum lycopersicum* (this species has two copies of this gene). In this species, one copy of the gene performs the canonical housekeeping function in the amino acid metabolism of catalysing the first reaction in the conversion of threonine to isoleucine (Rausher and Huang 2016). The second copy functions as an antinutritive defence against lepidopteran herbivores by depleting threonine in the insect's gut. Wild tobacco (*Nicotiana attenuata*) also contains a defensive copy. Rausher and Huang (2016) evaluated sequences of this gene in several Solanaceae species as well as *Nicotiana*, *Ipomea*, and *Petunia* species. They found that a single copy of *TD* underwent two duplications near the base of the Solanaceae (Rausher and Huang 2016). One copy retains the housekeeping function, whereas a second copy evolved defensive functions. Also, they revealed that positive selection occurred on the branch of the *TD2* gene tree subtending the common ancestor of the Nicotianoideae and Solanoideae. It also occurred within the Solanoideae clade but not within the Nicotianoideae clade. Finally, it occurred on most branches leading from the common ancestor to *S. lycopersicum* (Rausher and Huang 2016). According to the authors, *TD2* experienced adaptive substitutions for a period of 30–50 My (Rausher and Huang 2016). They suggested that the most likely explanation for this result is fluctuating herbivore abundances: When herbivores are rare, relaxed selection increases the likelihood that slightly disadvantageous mutations will be fixed by drift; when herbivores are common, increased selection causes the evolution of compensatory adaptive mutations (Rausher and Huang 2016).

A GWAS (genome-wide analysis association) study of herbivory in *A. thaliana* revealed the genetics of butterfly (*Pieris rapae*)-host herbivory (Nallu et al. 2018). *A. thaliana* GWAS resulted in a total of 90 associated SNPs in linkage disequilibrium with 389 genes. A subset of 12 well-supported candidate genes contained 3 or more associated SNPs each. Eight of these genes were functionally validated using mutants, showing increased larval weight gain and increased plant biomass eaten in knockouts versus control plants. This validated gene set includes both well-known and novel defence genes. For instance, the cytochrome P450 gene *CYP79B2* is involved in the conversion of tryptophan to indole-3-acetaldoxime, a precursor of indole glucosinolates and indole-3-acetic acid. Indole glucosinolates are essential secondary metabolites used for defence by *Arabidopsis* and other species of Brassicaceae (Nallu et al. 2018). The authors also found that genes *PROPEP1* and *PROPEP3*, which belong to the *At*Pep (endogenous danger peptides) gene family, are associated with activation of danger or HAMPs immunity in plants against both pathogen and herbivore attacks (Nallu et al. 2018) (Fig. 16.3).

Different Genes Responses to Biotic and Abiotic Stresses Combined and the Genetic Architecture of the Defensive Traits

Davila Olivas et al. (2017) studied the genetic architecture of plant responses before the attack of different herbivores (*Pieris rapae* and *Plutella xylostella*), a pathogen (*Botrytis cinerea*) and drought. Results show that 18 and 32 QTLs are linked to damage by *P. rapae* and *P. xylostella*, respectively. These QTLs contain candidate genes associated to plant resistance to insect herbivores, many genes explained only a small fraction of phenotypic variance, and some genes are induced by the two herbivores. For instance, in the case of *P. rapae*, the QTL 15 (chromosome 5) contains the *Myb29* and *Myb76* genes, and the closest significant SNP explains 4% of the phenotypic variance, whereas QTL 1 (chromosome 1) contains the *Bcat-1* and *Bcat-2* genes (Davila Olivas et al. 2017); the closest significant SNP explains 5% of the phenotypic variance. In the case of *P. xylostella* QTL 18 (chromosome 4) contained the *CP1* and *CP2* genes. *CP1* and *CP2* were induced by *P. rapae* and *P. xylostella* infestation. It has demonstrated that both *CP*1 and *CP*2 encode *CYSTEINE PROTEASE* enzymes (TAIR 10). In cotton *CP*2 has been implicated in

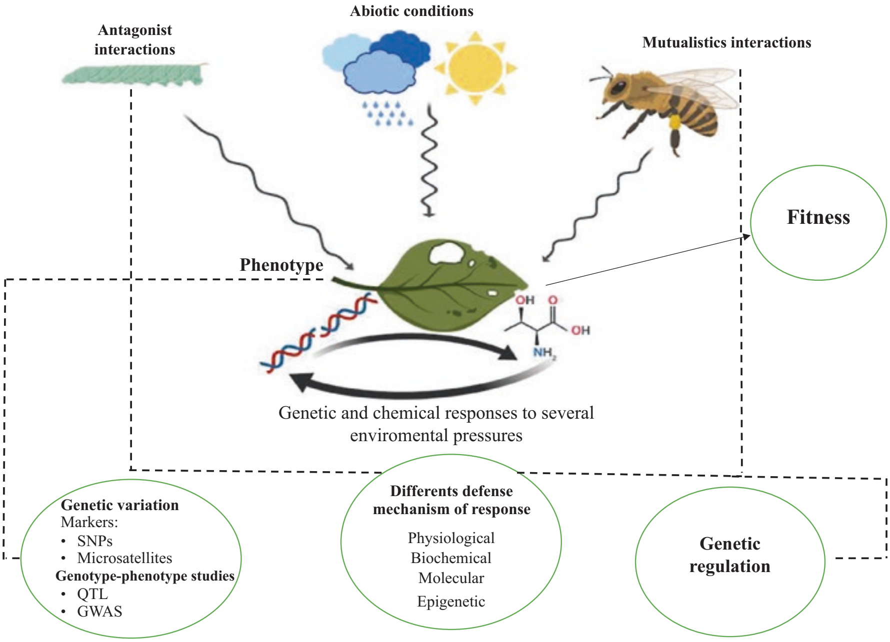

Fig. 16.3 Figure based on Alonso et al. 2019. Plants are able to defend themselves against their natural enemies by releasing chemical secondary compounds when they are being attacked. However, plants are part of complex interactions including pollinators and parasitoids, and they are also subject to changes in the abiotic conditions. Therefore, they have to “decide” how to face with its selective pressures. Different responses may be used by the plants as defence which include physiological, biochemical, molecular, and epigenetic mechanism. Advancement in technology and DNA sequencing provide new directions to study plant-herbivore interactions. For instance, association between phenotypes and genotypes can be reached by quantitative trait loci analysis (QTL) or genome-wide association analysis (GWAS)

increasing resistance against *Helicoverpa armigera* (Mao et al. 2011). Another example is QTL 32 (chromosome 5) which contains *XYP1*. The closest significant SNP explained 9% of the phenotypic variance. *XYP1* was induced by *P. rapae* and *P. xylostella* infestation and encodes a proteinase inhibitor/seed storage/lipid transfer protein. This type of protein has been implicated in antinutritional defences against insect herbivores (Heidel-Fischer et al. 2010).

Regarding gene responses to biotic and abiotic stresses combined, it was found that QTLs 19 and 25 are associated to the response to combined stresses drought + *Pieris* and *Botrytis* + *Pieris*, respectively. Interestingly, in the response to drought + *Pieris*, QTL 10 (chromosome 4) and QTL 19 (chromosome 5) contained the transcription factors MYC1 and AT5G50915. The two genes were induced by *P. rapae* infestation and slightly induced by drought (Davila Olivas et al. 2017). Natural variation in trichome density in *A. thaliana* is associated with genetic variation in MYC1 (Symonds et al. 2011). Several other transcription factors (e.g. MYC2, NAC) are major regulators of jasmonic acid- and abscisic acid-mediated

Table 16.1 Transcription factors families involved in plant defence

Stimulus	Transcription factor	Function	References
Ethylene responsive factor, RF family member that is induced by salt stress and drought	AP2/ERF	The roles of AP2/ERF TFs in biotic and abiotic stress responses as well as in developmental processes have been reported	Krishnaswamy et al. (2011)
The bHLH TFs acting in plant defence against pathogens	bHLH/	In animals, bHLH TFs mainly play roles in cell differentiation and neurogenic and myogenic processes. bHLH TFs in plants are involved in diverse biological processes as well	Seo et al. (2015)
Plays important roles in plant adaptation to environmental stress and development	ATAF1/2	Cup-shaped cotyledon	Jensen et al. (2013)
Responses to abiotic and biotic stress	bZIP	bZIP TFs regulate diverse biological processes such as seed formation, floral development	Kaminaka et al. (2006)
MYB TFs functioning in response to biotic and abiotic stresses as well as primary and secondary metabolism	MYB	MYB TF family is large and involved in controlling various processes like responses to biotic and abiotic stresses, development, differentiation, metabolism, defence etc.	War et al. (2012)
Defence responses, especially in sensing PAMPs or pathogen effectors and in downstream signalling	NAC	NAC TFs, response to biotic and abiotic stresses and in growth and development. For example, cold signals enhanced the proteolytic activation of a plasma membrane-bound NAC TF, NTL6, in *Arabidopsis thaliana*	War et al. (2012)
The play roles as transcriptome analyses revealed that many *WRKY* genes were induced following infection by pathogens	WRKY	WRKY TFs are involved in PAMP signalling downstream of mitogen-activated protein kinase (MAPK) cascades	Seo et al. (2015)
Pathogen attack and insect herbivory	MYC (MYC-12)	Leads to its interaction with JAZ and subsequent proteasomal degradation JAZ	Seo et al. (2015)
Cold stress, NTL6 (NTM (NAC with transmembrane motif1)-like 6) is induced and processed to relocate to the nucleus, activating *pathogenesis-related* (*PR*) gene expression	NTL6	Play crucial roles in diverse processes such as shoot apical meristem maintenance, lateral root formation	Tateda et al. (2014)

(continued)

Table 16.1 (continued)

Stimulus	Transcription factor	Function	References
Family coordinates stress signalling with wound healing. Different abiotic stresses: salt, drought, cold, ultraviolet B, heat, osmotic stress, as well as hormones such as ABA and JA	ERF108, ERF109, ERF110, ERF111	Heterodimerization turns the ERFs into highly potent cell division activators	Jan et al. (2008)
Pathogenesis-related (PR) proteins	TGA	Regulate defence gene expression for the generation of reactive oxygen species (ROS) and regulate specific plant responses to reactive oxylipins	Jan et al. (2008)
RDR proteins present in plants, role of RDR1, RDR2, and RDR6 for providing resistance against various biotic stresses endogenous small (sm) RNAs (primarily si- and miRNAs)	RDR 1, 2, 3, 4,5,6	Are important *trans-/cis*-acting regulators involved in diverse cellular functions	Seth and Axtell (2018)

responses, insect resistance, and drought responses (Dombrecht et al. 2007; Qi et al. 2015; Shinozaki and Yamaguchi-Shinozaki 2007; Table 16.1). Finally, QTL 3 (chromosome 1) contains the transcription factor AT1G19210, and the SNP with the highest effect within this QTL explained 6% of the phenotypic variance. AT1G19210 was induced on *P. rapae* infection, drought, jasmonic acid and abscisic acid. This transcription factor has been implicated in tolerance to drought and freezing and resistance to necrotrophic fungi (Davila Olivas et al. 2017). Thus, the authors revealed that different genetic components control resistance to the two caterpillars. There is limited overlap in the quantitative trait loci (QTLs) underlying resistance to combined stresses by drought plus *P. rapae* or *B. cinerea* plus *P. rapae* and *P. rapae* alone (Davila Olivas et al. 2017).

R Genes Family and *Mi-1.2* Gene Are the Only Genes That Have Been Described in a Gene-for-Gene Interaction Between Plants and Their Natural Enemies

In plants, resistance (*R*) genes play a key role in their remarkable immune responses (Kourelis and van der Hoorn 2018). *R* genes are usually dominant (but sometimes recessive) genes that provide full or partial resistance to one or more pathogens

(Kourelis and van der Hoorn 2018). *R* genes exist in natural plant populations and have been used by humankind since early crop domestication (Kourelis and van der Hoorn 2018). Selection during domestication favoured dominant *R* genes providing full resistance, but recessive *R* genes and *R* genes that provide partial resistance may provide more durable resistance (Kourelis and van der Hoorn 2018). Most identified *R* genes are polymorphic in plant populations, which led to their initial characterization and use in plant breeding programmes. However, individual plants have up to a few hundred *R* gene analogues that make no identified contribution to resistance (Kourelis and van der Hoorn 2018). Many of these R gene analogues are also fixed in plant species and are thought to contribute to nonhost resistance (Schulze-Lefert and Panstruga 2011). Plant *R*-genes are involved in gene-for-gene interactions with pathogens, and they may undergo coevolutionary arms races in which plant specificity and pathogen virulence or insect infestation continually adapt in response to each other (Bergelson et al. 2001). The *R*-genes evolution is shaped by natural selection for resistance to different insect species but especially for species of aphids (Bergelson et al. 2001; Howe and Jander 2008; Michelmore and Meyers 1998; Smith and Boyko 2007). Evidence points that the products of *R* genes mediate resistance to phloem-feeding insects in several monocot and dicot crop species (Smith and Boyko 2007; Howe and Jander 2008).

In tomato, the *Mi-1.2* gene provides resistance to some isolates of *Macrosiphum euphorbiae* (potato aphid) and *Bemisia tabaci* (silverleaf whitefly), but not to *Myzus persicae* (green peach aphid) (Nombela et al. 2003). *Mi-1.2* confers resistance to multiple species of arthropods and nematodes (Nombela et al. 2003). The LRR (leucine-rich repeat) region of *Mi-1.2* signals programmed cell death, and one model proposes a gene-for-gene interaction between *Mi-1.2* and aphid elicitors, similar to plant-pathogen interactions (Hwang and Williamson 2003). Other studies suggest NBS-LRR involvement in aphid resistance in other crops (see Smith and Clement 2012).

Elicitors Induce Defensive Genes

Adding regurgitant of Colorado potato beetle (*Leptinotarsa decemlineata*) to wounded leaves of potato plants elicits the expression of 73 genes when compared to leaves only wounded (Lawrence et al. 2008). An analysis of five differentially expressed genes between treatments found that genes involved on induction are related to secondary metabolism and stress. One induced gene encodes an aromatic amino acid decarboxylase, responsible for the synthesis of the precursor of 2-phenylethanol, which is recognized by the predator of *L. decemlineata* (*Perillus bioculatus*). Also, 3 out 16 type 1 and type 2 proteinase inhibitor clones present on the potato microarray were repressed by application of CPB regurgitant to wounded leaves. Given that proteinase inhibitors are known to interfere with the digestion of proteins in the insect midgut, repression of these proteinase inhibitors by CPB may inhibit this component of the plant's defence arsenal (Lawrence et al. 2008).

Therefore, the authors suggest that beyond the wound response, CPB elicitors play a role in mediating the plant-insect interaction.

Phytohormones as Regulators of Defensive Response

Herbivorous insects produce oral secretions containing compounds that elicit plant responses (Bonaventure et al. 2011; Stam et al. 2014). The chemical nature of active compounds is remarkably diverse, including small organic compounds such as benzyl cyanide, fatty acid-amino acid conjugates, and proteins such as β-glucosidase (Stam et al. 2014). The recognition of herbivore elicitors by plant receptors initiates a cascade of responses, including changes in plasma membrane potential and activation of networks of MAP kinases and phytohormones (Stam et al. 2014) (Fig. 16.2). In particular, this response to arthropod herbivory triggers reactive oxygen species and signal cascades involving jasmonic acid (JA), salicylic acid (SA), ethylene, abscisic acid, cytokinins, auxins, and gibberellic acid that result in a downstream production of direct and indirect defence proteins such as *R* proteins (Smith and Clement 2012; Kourelis and van der Hoorn 2018). Defence response gene upregulation via JA and other pathways results in the production of many defence allelochemicals (Chen 2008; Smith and Clement 2012) (Fig. 16.2). Less, however, is known on arthropod-induced expression of plant metabolism genes, but sparse evidence indicates that some of these genes are downregulated the initial hours after the onset of arthropod herbivory and subsequently upregulated during ensuing days (Smith and Boyko 2007; Smith and Clement 2012).

The expression of a gene is determined by the cis-acting DNA elements located in the vicinity of the gene and the trans-acting protein factors that interact with them. These cis-acting elements are concentrated in a relatively small promoter region of a few hundred nucleotides upstream of the transcriptional start site; other regulatory sequences are located at a distance of several thousands of nucleotides from the gene (Memelink 2009). Several cis-acting elements in various gene promoters that mediate phytohormones (such as jasmonate) responsiveness have been identified. The most common jasmonate-responsive promoter sequences are the GCC motif and the G-box. Besides several other jasmonate-responsive promoter elements have been reported (Memelink 2009).

The most studied phytohormone, jasmonic acid (JA), and its cyclic precursors and derivatives are collectively referred to as jasmonates (JAs) and constitute a family of bioactive oxylipins that regulate plant responses to environmental and developmental cues (Wasternack 2007) (Fig. 16.2). These signalling molecules affect a variety of plant processes including fruit ripening (Creelman and Mullet 1997), root elongation (Staswick et al. 1992), response to wounding (Zhang and Turner 2008) and abiotic stresses, defence against insects (McConn et al. 1997), and necrotrophic pathogens (Thomma et al. 1999). Also, there is evidence that the jasmonates 12-oxo-phytodienoic acid (OPDA), JA, and methyl-jasmonic acid (MeJA) act as active signalling molecules to herbivory (Wasternack 2007; Memelink 2009).

Evidence on the role of jasmonates in plant-insect interactions derives from the analysis of mutants that fail to perceive JA/MeJA (Howe and Jander 2008) (Fig. 16.2). Mutants that are defective in the *Coronatine insensitive* 1 (*COI1*) gene are impaired in all jasmonate-signalled processes and highly susceptible to a wide range of arthropod herbivores (*reviewed* in Howe and Jander 2008) (Fig. 16.2).

Once herbivory occurs, JA is produced via the octadecanoid pathway. In *Arabidopsis*, the enzyme jasmonoyl isoleucine conjugate synthase 1 (JAR1) activates JA by conjugating it to the amino acid isoleucine (Ile) to form JA-Ile (Stam et al. 2014). Within the JA signalling pathway, two branches have been identified which act antagonistically (Pieterse et al. 2012). The MYC2 branch positively regulates the expression of wound-inducible JA-responsive marker genes such as vegetative storage protein 2 (*VSP2*) and lipoxygenase 2 (*LOX2*). In the ethylene (ET) response factor (ERF) branch of the JA pathway, JA and ET synergistically induce the expression of JA/ET-responsive transcription factors, including ERF1 and octadecanoid-responsive *Arabidopsis* 59 (ORA59), which positively regulate JA/ET-responsive genes such as plant defensin 1.2 (*PDF1.2*) (Stam et al. 2014). The ERF branch is mainly involved in induced defence against necrotrophic pathogens, whereas the MYC2 branch mediates defence against herbivorous insects (Stam et al. 2014).

Salicylic acid (SA), a benzoic acid derivative, is also an important phytohormone involved in regulation of plant defence (War et al. 2012). It is an essential endogenous plant growth regulator involved in a wide range of metabolic and physiological responses in plants, including defence and plant growth and development (Rivas-San and Plasencia 2011). Responses to SA depend on a regulatory protein called Non-Expressor of Pathogenesis-Related Genes1 (*NPR1*). The *NPR1* gene is activated through redox pathways by SA accumulation and is translocated to the nucleus. However, it does not bind to DNA directly but acts through transcription factors (War et al. 2012). SA induces greater defence against insects that pierce or suck plants rather than chewers (War et al. 2012). Moreover, production of reactive oxygen species (ROS) by SA pathway is thought to induce resistance in plants against insect pests (e.g. tomato and *H. armigera;* Peng et al. 2007).

Initially it was thought that plant damage by arthropods' chewing mouthparts would elicit JA-based transcriptomes and that arthropods with piercing-sucking mouthparts would induce JA-SA-based transcriptomes (Smith and Clement 2012). Nonetheless, it has been demonstrated that JA-SA signalling and JA-SA crosstalk are induced by both types of herbivores' feeding habits (Smith and Boyko 2007; Smith and Clement 2012). Crosstalk between phytohormonal signalling pathways may permit herbivores to manipulate plant defences in their interest (Stam et al. 2014). In example, feeding by *Manduca sexta* caterpillars induced an ET burst and suppressed nicotine accumulation in tobacco plants (Kahl et al. 2000). It has been hypothesized that by activating the SA signalling pathway, phloem feeders suppress the JA-dependent defences to which phloem feeders are more sensitive (Stam et al. 2014; Zarate et al. 2007). Recent studies indicate an interference between SA with JA-inducible defences against chewing insects (Lu et al. 2014), although not always phloem-feeding insects interfere with defences induced by chewing herbivores, perhaps due to density effects or to differences between species (Stam et al. 2014).

Ethylene is another important phytohormone that plays an active role in plant defence against many insects (van Loon et al. 2006). Ethylene signalling pathway participates directly and indirectly on induced plant defence against herbivores and pathogens *(*van Loon et al. 2006). For instance, infestation by *Alnus alni* induces the emission of ethylene and the release of various volatiles in *Alnus glutinosa* L. leaves (War et al. 2012).

The hormone systemin (Pearce et al. 1991; Ryan and Pearce 1998) plays a regulatory role in many aspects of the plant life, including growth, development, fertilization, and interactions with symbiotic organisms (Wang et al. 2018). It is an amino acid peptide derived from a larger precursor protein. It was proposed that systemin functions spreading signal that triggers the systemic defence responses observed in plants after wounding or attack by herbivores (Pearce et al. 1991). A leucine-rich repeat receptor kinase (LRR-RK) is identified as the systemin receptor 160 (SR160) (Torii 2004). SR160 is a tomato homologue of Brassinosteroid Insensitive 1 (BRI1), which mediates the regulation of growth and development in response to the steroid hormone brassinolide (Szekeres 2003). Wang et al. (2018) demonstrated that the perception of systemin depends on a pair of distinct LRR-RKs (leucine-rich repeat receptor kinase) called SYR1 and SYR2. SYR1 acts as a genuine systemin receptor that binds systemin with high affinity and specificity and the authors showed that the presence of SYR1 is important for defence against insect herbivory (Wang et al. 2018).

Epigenetic Regulation in Response to Defence

Environmental factors may modify the plant's regulation of individual genes through different mechanisms (Fig. 16.1) such as DNA methylation, lysine methylation in histones, histone acetylation, histone phosphorylate, RNA interference: RISC; siRNA, microRNA, transposition of mobile elements: insulators, promoters, enhancers, transposons (*see* Ramirez-Prado et al. 2018). Different important factors in gene regulation are linked to plant defence and transcriptional reconfiguration (Table 16.1). Epigenetic regulation is an important mechanism of immediate response after the attack of herbivores or pathogens, for instance, the signalling cascades in which participate two important phytohormones, jasmonic acid (JA) and salicylic acid (SA). The silencing sequences of transposable elements within heterochromatin are probably a genomic defence strategy. Indeed, recent evidence demonstrates that plant defence gene expression also involves DNA methylation and histone modifications that are closely linked to the dynamical chromatin states (Law and Jacobsen 2010; Lämke and Bäurle 2017; Ramirez-Prado et al. 2018). For instance, a major class of *R* proteins are the NLR immune receptors that mediate ETI to various pathogens (Espinas et al. 2016). *NLR* genes often form gene clusters in the genome that contain repetitive sequences and TEs (Meyers et al. 2003). The repetitive nature of *NLR* gene clusters is thought to facilitate rapid expansion and sequence diversification of these genes, possibly by promoting unequal recombination (Friedman and Baker 2007; Espinas et al. 2016). It is well documented that TEs inserted in the promoter region often regulate neighbouring genes in both animals and plants by changing their epigenetic states (Slotkin and Martienssen 2007). A recent report shows that TEs in intronic regions can regulate *NLR* expression in *Arabidopsis* (Eulgem et al. 2007). *Arabidopsis RPP7* encodes a CC-NBS-LRR class of NLR that confers resistance to downy mildew, *Hyaloperonospora arabidopsidis* (Hpa) (Eulgem et al. 2007).

Transcriptions Factors as Regulators of Defensive Traits

Regulation of gene expression is given by transcription factors; they are protein sequences that specifically bind to cis-regulatory DNA sequences and may have activities as corepressors or coactivators (Petrillo et al. 2015). The control phytohormonal and gene expression in plants crosstalk to herbivory result in transcriptional responses that have a degree of specificity (Stam et al. 2014). Transcriptional responses of the plants response depend on the feeding guild of the attacker and the phytohormonal signal signature that the attacker induces (Stam et al. 2014). Recent studies in *Nicotiana attenuata* showed that aphids suppressed more genes than chewing herbivores did and aphids upregulated the expression of SA-dependent genes and suppressed the expression of JA-mediated genes (Heidel and Baldwin 2004). This review lists the recent findings related to the responses defence function of plants' transcription factors and the regulations of expression (Table 16.1).

Conclusions and Future Directions in the Genomics Era

Plant's genotype determines not only constitutive plant traits but also inducible plant responses, such as the production of metabolites or structural changes. The extent to which constitutive or inducible traits affect plant-insect interactions influences the relative importance of the inducible and constitutive phenotypes concerning their impact on community dynamics (Bidart-Bouzat and Kliebenstein 2011). In this brief review, have attempted to call the attention to some remarkable studies on the molecular basis of defensive traits in plants. These studies deepen our understanding of plant defence genes and the role of natural selection in shaping the genetic variation and structure of natural, and cultivated, plant populations. Furthermore, it is important to stress the need of more studies on the different genetic responses to different stresses, to elucidate the physiological mechanisms activated by the plants. Progress in molecular biology (functional genomics, genome-wide association studies, and QTL studies), analytical chemistry (metabolomics) is rapidly aiding to widen our understanding of the mechanisms linking physiological responses to ecological interactions. Genome-wide data offer many advantages over sparser sets of genetic markers (Savolainen et al. 2013). It is now possible to detect the signature of selection across the genome and if putative genes are associated with herbivory. As before, the understanding of the genetic basis of plant defence traits is today important in the face of climate change, crop production and pest control. Likewise, genomic tools may help in designing research strategies to combine genomics and field experiments to examine the genetics of local adaptation (Savolainen et al. 2013). Thus, we can tackle the analysis of phenotypic patterns generated by spatially varying selection, genetic mapping, and the genetic architecture of defence adaptive traits (Savolainen et al. 2013).

The recently identified interactions among signalling pathways involved in plant growth with defence signalling networks and the role of the phytohormones provide a good starting point to test hypotheses on the regulation of ontogenically driven defence responses (Stam et al. 2014). Fundamental research on crosstalk among growth hormones and defence responses has mostly been performed with models' organisms such as *A. thaliana*, *Solanum lycopersicum*, and *Nicotiana attenuata*. The elucidation of the genetic architecture of defence traits in other, non-model, plant species is, however, welcomed to uncover the manifold evolutionary phenotypic route taken by interacting plants and herbivores.

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Chapter 17
Ecological Genomics of Plant-Insect Interactions: The Case of Wasp-Induced Galls

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Abstract Plant-insect interactions are central to understand ecological and evolutionary dynamics that shaped phenotypes and genotypes. Although the interaction between host plants and insect herbivores has been widely assessed, the molecular mechanisms behind it are largely unknown. Here, we discuss the significance of the ecological genomics for the study of nonmodel species in the context of specialized herbivore interactions. First, we provide an overview of ecological genomics and review functional genomic studies addressing plant responses to herbivores. Second, using the oak-wasp interaction as a framework, we addressed the molecular mechanisms involved in the response of host plants to specialized phytophagous insects. We present a functional study of gene expression along the development of oak galls induced by cynipid insects. The transcriptomic profiles depicted show changes in gene regulation related to metabolism and cell cycle, which are consistent with the developmental trajectory of growing larvae. These findings suggest phenotype

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manipulation of the host plant by the wasp larvae and support the adaptive role of galls as a life history trait of insects. Our study provides insight of how the ecological genomics approach can contribute to elucidate the genetic bases of organism's response to their environment and thus help to better understand species interactions and adaptation. In order to unravel the genetic control of natural variation, we suggest that future research should encompass applied ecology and evolution, molecular biology, and bioinformatic and genomic tools.

Keywords Cynipid insects · Ecological genomics · Oak-wasp interaction · Plant-insect interactions · Specialized phytophagous insects · Wasp-induced galls

One of the major challenges in biology is to connect phenotypes with genotypes (Pavey et al. 2012) and identify the factors that drive their variation in natural populations. Meeting this challenge entails an integrative approach involving different analytical techniques and a combination of disciplines such as evolutionary and functional ecology, and population and quantitative genetics. This interdisciplinary approach has given rise to the field of ecological genomics (Feder and Mitchell-Olds 2003), which incorporates tools that are usually used for the study of genomes from a single model organism into the study of specific genes across nonmodel species (Ungerer et al. 2008; Straalen and Roelofs 2012). This new discipline provides a comprehensive framework in the study of complex processes such as biological interactions at different hierarchical levels, addressing from phenotypic variation and the mechanistic bases underlying an interaction (e.g., pollinators are responsible for the variation in MYB genes linked to color and flower scent; Yuan et al. 2013) to the evolution of species (e.g., plant-herbivore interaction drives the coevolution of host plants and phytophagous insects; Ehrlich and Raven 1964).

Consumer-resource interactions include a variety of antagonistic and specialized associations such as prey-predator, plant-herbivore, and host-parasite interactions. Plant-animal interactions, especially the associations of plants with phytophagous insects, are one of the most widespread and complex types of interactions among organisms, involving almost a half of the known species of the planet (Strong et al. 1984; Roskov et al. 2018). In recent years, genomic studies of the interaction of plants with their natural enemies have contributed to the elucidation of the mechanistic bases of the variation, regulation, and evolution of the chemical defenses of plants (Züst et al. 2012; Zhang and Liu 2015) that drive the diversification of plant and insect lineages (e.g., Edger et al. 2015). The study of the ecological genomics of the species-specific response of plants to herbivores may help to understand the common physiologic and metabolic pathways of organisms and may simultaneously contribute to elucidate the unique molecular mechanisms underlying specialized interactions.

In this chapter, we introduce the significance of the ecological genomics approach for the study of nonmodel organisms. We use one of the most specialized herbivore associations, the oak-cynipid interaction, as a model system for understanding the molecular bases of the responses of plants to their biotic environment. First, we provide an overview of the main approaches used in ecological genomics to assess gene expression and function and review the functional genomics approach for analyzing plant responses to phytophagous insects. We highlight key aspects of the induction and development of oak galls by cynipid wasps. Then, based on the functional analysis of gene expression, we examine the induced response of *Quercus castanea* to infestation by *Amphibolips michoacaensis*. We focus on patterns of differential expression of genes related to development, nutrients, and defense in the host plant. Finally, we discuss the future directions in the study of biological interactions in the field of ecological genomics.

Overview: Ecological Genomics and the Functional Approach for the Study of Plant-Insect Interaction

The theoretical basis of ecological genomics relies on the integration of the analyses of genomes and the ecological relevant genes applied to multiple species, for understanding the relationship between the organisms and its biotic and abiotic environments (Ungerer et al. 2008; Straalen and Roelofs 2012). Genomic variation can be considered at the genetic, epigenetic, and transcriptional levels, addressing the structure, function, and evolution of genes and genomes (Griffiths et al. 2005). Broad analytical approaches for the study of genomic variation include (1) the genotyping of molecular markers, (2) comparative gene regulation and genome structure, and (3) genetic manipulation to test gene function (Bengston et al. 2018). Most of the molecular studies that seek to understand the complex genotype-phenotype relationships integrate genomic information on a global scale (genome-wide) with measures of traits. In Table 17.1, we provide an overview of the main molecular methodologies used under the ecological genomics approach. Among these approaches, the study of the molecular architecture of traits is useful for the identification of genes, pathways, and networks that underlie common responses of organisms to their abiotic and biotic environment (e.g., Civelek and Lusis 2014). Functional genomics is a field of molecular biology that uses the vast amounts of data generated by genomic and transcriptomic analyses such as genome sequencing, to describe gene (and protein) functions and interactions (Gibson and Muse 2009). A key characteristic of functional genomics studies is their genome-wide approach involving high-throughput methods, rather than a more traditional "gene-by-gene" approach. Functional genomics studies specifically focus on the dynamic aspects, such as gene transcription and translation, and regulation of gene expression and protein-protein interactions as opposed to the static aspects of the genomic information, such as DNA sequences or structures (Pevsner 2015). Through this approach, a wide range of biological questions can be addressed, including: When and where are genes expressed? How do gene expression levels differ among cell

Table 17.1 Ecological genomic approaches

Ecological/genomic approach	The rationale	Examples of specific methodologies	Examples in plant interactions
Genome-wide association (GWAS) approach: Scan the entire genome searching for common genetic variation	Linkage between particular traits with genes or genome regions will be exhibit when examine single-nucleotide polymorphisms (SNPs) across genome This approach needs no a priori knowledge of gene association with traits	Genome-wide genotyping or sequencing	Poland et al. (2011), Horton et al. (2014), and Wen et al. (2014)
Candidate gene approach: Focuses on allelic-specific biologically relevant regions of the genome to associate genetic and phenotype variation with pre-specified genes	A mutation in the regulatory region of a given gene could result in a different pattern of expression and function, being causal candidate for the phenotypes' variation. This approach is based on a priori knowledge of functional impact of genes on traits	Quantitative trait locus (QTL) mapping Transcription profiling (microarrays, SAGE, next generation sequencing) Reverse genetic (heterologous expression, gene silencing, and overexpression)	Philippe et al. (2000), Kliebenstein et al. (2002), Halitschke and Baldwin (2003), Broekgaarden et al. (2010), and Holeski et al. (2010)
Genetic and population approach (phenotypic and genetic characterization): Test wheatear phenotypes are the result of genetic differences within and among populations and how the environment shapes both genetic and phenotypic variation	A phenotype recaps genotypic expression and inherited epigenetic cues influenced by environmental factors and their interaction between each one. By assessing the dynamics of phenotype/genotype/environment within and among populations, phenomena as variation, adaptation, speciation, and population structure are expose This approach provides the insights of trait relevance for particular biological process	Experimental and field studies in natural populations Polymerase chain reaction (PCR) DNA cloning and isolation Microsatellites	Gómez-Gómez et al. (1999), Kliebenstein et al. (2001), and De-la-Mora et al. (2018)

types and states? What are the functional roles of different genes (or proteins), and what processes are they involved in? Most of these questions have been assessed across diverse plant taxa, testing their responses to herbivores and pathogens (e.g., Martin et al. 2003; Thompson and Goggin 2006; Holliday et al. 2017; Wróblewski et al. 2018; Rendón-Anaya et al. 2019). In recent years, the interaction of plants with their natural enemies has provided a fruitful framework for understanding when and how genes are involved in the responses of organisms to their biotic environment.

The responses of plants induced by phytophagous and pathogens involve hundreds to thousands of differentially regulated genes. The functional genomics approach has contributed to elucidation of plant responses to insect herbivores (Box 17.1). These responses involve (1) phytohormone-mediated signal transduction (Zheng and Dicke 2008; Luo et al. 2019), (2) specific proteins that trigger defensive/immune reactions (e.g., Zipfel 2008; Buron-Moles et al. 2015), and (3) transcription factors regulating defense-associated proteins/genes (Eulgem 2005; Rushton et al. 2010). Recent advances in this field have led to the rapid identification of key genes involved in induced plant responses in different nonmodel species, illustrating the value of this analytic approach for understanding the ecological genomics of plant-insect interactions.

Box 17.1: Empirical Evidence of the Functional Genomics Approach for the Study of Plant Responses to Phytophagous Insects

Phytohormone-Mediated Signaling

Three major signal transduction paths are responsible for induced responses of plants to wounding. The shikimic acid pathway, the ethylene pathway, and the jasmonate pathway, characterized by the phytohormones salicylic acid (SA), ethylene (ET) and jasmonic acid or jasmonate (JA), respectively (Dicke and Van Poecke 2002; Traw and Bergelson 2003; Heil et al. 2004; Howe 2004). Of these phytohormones, JA seems to play a key role in plant-insect interactions. For instance, transcriptomic studies in *Hyoscyamus* sp. (Solanacea) have shown that the synthesis of defense-related compounds is jasmonate-dependent (Moyano et al. 2003). Also, empirical evidence derived from microarrays and transcription profiling studies from *Arabidopsis* sp. indicates that the attack of herbivore insects directly reprograms metabolism, inducing transcriptional changes on JA-related genes (e.g., De Vos et al. 2006; Broekgaarden et al. 2007).

Transcription Factors Regulating Defense Response

Gene expression of the *defense cascade* involves the spatiotemporal transcription regulation of metabolic pathways, controlled by a complex network of many regulatory proteins known as transcription factors. These proteins bind to specific cis-regulatory DNA sequences to control the rate of transcription to messenger RNA. The transcription factors recognize the promoters of target genes and activate or repress their expression in response to developmental and/or other environmental cues (Yan et al. 2018). Major response of plants to injury relies in the production of secondary metabolites. Wide-scale studies assessing transcription for secondary metabolism conducted in model (e.g., *Arabidopsis* sp. and *Medicago truncatula*; May 2002) and nonmodel species (e.g., *Gossypium hirsutum*; Salih et al. 2016) have documented that family proteins functioning as transcription factors are usually highly conserved and widely diversified across plant taxa. A good example is the WRKY transcription factor superfamily which occurs in most of the botanical families. This transcription factor superfamily is involved in many plant processes including plant responses to wounding (Zhang and Wang 2005).

Cynipids and the Induction of Oak Galls

Many arthropod species are plant herbivores that develop in a host for part (e.g., the Hessian fly *Mayetiola destructor*, Prestidge 1992) or all their life cycle (e.g., the spider mite, *Tetranychus urticae*, Orsucci et al. 2017). Some arthropod species living in plants can even alter the morphological and physiological attributes of their host by inducing different types of abnormal structures in several tissues. These induced structures known as galls (Fig. 17.1) serve as both the habitat and food source for the gall-inducing arthropods, which usually spend their larval phase within the gall (Raman et al. 2005). Gall-inducing insects include gall wasps, gall midges, gall flies, Agromyzidae, aphids, and goldenrod gall flies, among many others (Price 2005). Gall wasps (Cynipidae: Hymenoptera) comprise ˜1300 species distributed worldwide (Lijeblad and Ronquist 1998). The Cynipidae family is unique within the Cynipoidea superfamily since all its members are specialized phytophagous insects, either inducing the formation of galls or simply inhabiting the existing ones. Most gall-inducing wasps (approximately 87% of species) are associated with oaks (Abrahamson et al. 1998). Oaks (*Quercus* spp.) are cosmopolitan trees native to the Northern hemisphere extending from cool temperate to tropical latitudes (Valencia 2004). *Quercus* includes almost 600 deciduous and evergreen species, which are all susceptible to wasp infestation. This long-term, highly specialized association between oaks and cynipids has been attributed to the partitioning of spatiotemporal niches and the selective pressure of parasitoids and predators (Cornell 1983; Askew 1984), which is linked to the chemical composition of oaks based on phenolic compounds (Box 17.2).

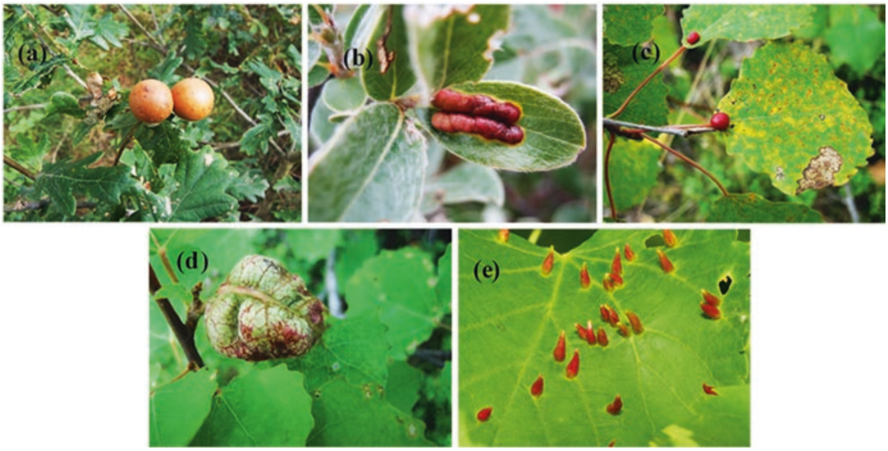


Fig. 17.1 Morphotypes of plant galls induced by arthropods. (**a**) Wasps (Hymenoptera: Cynipidae) on *Quercus* sp. (Fagaceae), (**b**) sawflies (Hymenoptera: Tenthredinidae) on *Salix glauca* (Salicaceae), (**c**) midges (Diptera: Cecidomyiidae) on *Populus tremula* (Salicaceae), (**d**) aphids (Hemiptera: Aphididae) on *Populus* sp. (Salicaceae), and (**e**) mites (Acari: Eriophyidae) on *Tilia* sp. (Malvaceae)

Box 17.2: Chemical Defenses of Oaks

Oak trees are known to produce several classes of phenolic compounds such as flavonoids, tannins, and lignin, among others (Feeny 1976; Forkner et al. 2004; Moctezuma et al. 2014). Phenolic compounds derived from the amino acid L-phenylalanine via deamination by L-phenylalanine ammonia-lyase (PAL). Different classes of phenols are synthetized through different biosynthetic routes that can be organized into a “core” phenylpropanoid pathway, from phenylalanine to an activated (hydroxy)cinnamic acid derivative via the actions of PAL, cinnamate 4-hydroxylase (C4H), and 4-coumarate coenzyme A ligase (4CL), and the specific branch pathways for the formation of monolignols/lignin, coumarins, benzoic acids, stilbenes, and flavonoids/isoflavonoids.

Phenols occur in leaves and oak galls (mostly restricted to outer layers; Nyman and Julkunen-Tiitto 2000; Allison and Schultz 2005). When ingested phenolic compounds can have a deterrent or toxic effect on herbivores. Phenols toxicity in insects is thought to result from the production of high levels of reactive oxygen species (Barbehenn and Constabel 2011). In some *Quercus* species (e.g., *Q. robur*), these compounds show seasonal variation and a differential distribution between gall and nongalled tissues (the gall tissues have a higher phenol levels than nongalled tissues; Hartley 1998). Oak phenols have been implicated in the evolution of feeding habits of herbivores, shaping the abundance and richness of leaf-chewing insects (Feeny 1976). The concentration and variation of oak phenols often explain the variation in herbivore community structure (Forkner et al. 2004).

The galls induced by cynipids comprise highly differentiated cell layers, including nutritive, spongy, and epidermal tissues (Brooner 1992). Each of these tissues undergoes several changes during the different phases of gall development, which are associated with the developmental changes of the galls (Box 17.3). When a gall is induced, significant changes in cell metabolism and differentiation occur. The main metabolic changes during gall development include changes in the biosynthesis of carbohydrates and secondary metabolites (Brooner 1992; Tooker et al. 2008). For instance, at the early stages of the gall growth phase, the concentration of sugar increases due to an increase in number and size of amyloplasts (i.e., organelles that synthetize and store starch granules) (Box 17.4). Similarly, the chemical profile of gall tissues is usually modified in response to infestation of the parasite larva. For example, comparison of ungalled leaves and oak galls induced by cynipid wasps of the genera *Andricus* and *Neuroterus* revealed that the concentration of defense-related phenolic compounds decreases or increases, respectively, in galled tissues (Hartley 1998). The metabolic changes that increase the sugar content of gall tissues and, hence, their nutritional quality (the nutrition hypothesis, Bronner 1977; Price et al. 1986), as well as the changes involved in the synthesis of defensive compounds such as phenolics (the enemy hypothesis; Cornell 1983, Price et al. 1986)

Box 17.3: Development of Cynipid Larvae and Galls

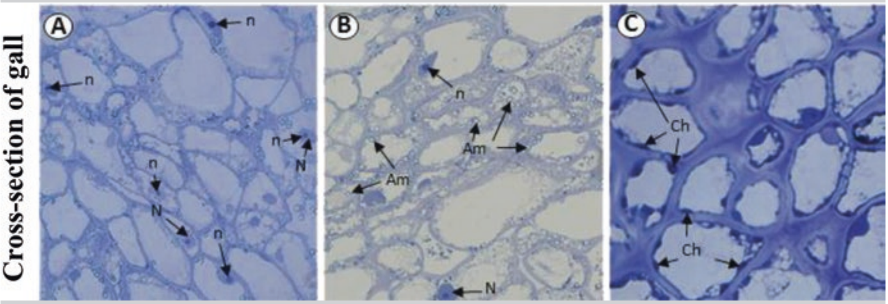
Wasp development comprises four developmental stages: egg, larva, pupa and adult. From the moment that a female wasp lays eggs until the adult emerges from the gall, parasite growth is linked to the gall (Price et al. 1987; Stone and Schönrogge 2003). The morphological properties of galls are strongly associated with the changes that larvae undergo during the immature stages (larval instars, prepupal and pupal phase) (Stone et al. 2002). Hence, histological changes of galls (e.g., cell division) or changes in appearance (e.g., color or toughness) are indicative of the general patterns of the development of the parasite. Wasp galls comprise, from the inside out, three highly differentiated cell layers; the nutritive, spongy and epidermal tissues (Brooner 1992). Each gall layer experiences significative changes during three different phases of development, which are characterized as follows. (1) The initiation phase starts with the laying of eggs in the meristematic tissue of the host plants (Rey 1992). During this phase, the cells enclosing the eggs become necrotic and the proliferation of the adjacent cell layers begins. (2) The growth phase is distinguished by the differentiation of nutritive cells surrounding the larva. These cells increase in number (i.e., hyperplasia) and size (i.e., hypertrophy) and constitute the food source for the growing larva (Meyer and Maresquelle 1983; Brooner 1992; Brooks and Shorthouse 1997). (3) At the maturation phase the larva stops growing and pupates, cell division decreases, and most gall tissues become lignified and eventually desiccate. Lignification makes the tissue unusable for other herbivores, and in some species, the onset of lignification determines when the galled organ (e.g., leaves or acorns) is shed from the host (Stone et al. 2002; Csóka et al. 2005).

Box 17.4: Histological Characteristics of Galls Induced by *Amphibolips michoacaensis*

Hernández-Soto et al. (2015) presented the histological description of oak galls induced by *Amphibolips michoacaensis* during growth. The authors sampled galls of different sizes that contained larvae (n = 80), identified three layers of tissue (i.e., nutritive, spongy, and epidermal cells) and characterized cytological changes according with phases of development as follows:

In small galls at early growth phase (~7 mm diameter), three layer of tissue were well differentiated. Nutritive cells were characterized for the presence of hypertrophied nucleus and nucleolus; numerous amyloplasts were found in spongy cells and thickened cell walls with chloroplasts in epidermal tissue. Medium galls (14–24 mm) at the intermediate growth phase showed a gradual degradation of the nucleus and nucleolus in nutritive cells and no chloroplast in the epidermal tissue. Large galls (35–56 mm) at the late growth phase exhibited transitional properties between the growth and mature phases. In galls of 36–56 mm, the nutritive tissue rarely included the nucleus and nucleolus, and the cell morphology was not different from the spongy tissue. In large galls, most of the spongy cells were empty and had lignified walls, and there was no difference between the three gall layers.

In the following figure, it is shown a cross-section of a gall induced by *Amphibolips michoacaensis* during the early growth phase (small gall). Single larval chamber (Lc), nutritive (N), spongy (S), and epidermal (E) cells (color photo at the left). (**a**–**c**) Light micrographs of the different gall layers. (**a**) Nutritive cells present hypertrophied nucleus (N) and nucleolus (n). (**b**) Spongy cells present numerous amyloplasts (Am). (**c**) Epidermal cells show numerous chloroplasts (Ch). (Modified from Hernández-Soto et al. 2015)



have been invoked to explain the adaptive significance of gall induction as a life history trait of insects (Stone et al. 2002). Although most of these metabolic changes have been examined in cytological and chemical studies across several plant taxa, the precise mechanisms implicated in the formation and control of the induced galls, including their genetic regulation, are poorly understood (Raman and Dhileepan 1999; Wool et al. 1999; Redfern 2011).

Functional Genomics of the Oak-Cynipid Interaction: Gene Expression in Wasp Galls of *Quercus castanea*

Here, we discuss the molecular bases of the oak-cynipid interaction. We used a functional genomics approach to analyze the differential gene expression in the galls of *Quercus castanea* induced by the cynipid wasp *Amphibolips michoacaensis*. We present the main patterns of gene regulation related to (1) gall development, (2) nutrients, and (3) defense. To examine the genetic control of the nutritional and defensive patterns of galls, we focused on genes associated with amylase and phenolic compounds as well as with the hydrolytic and oxidative enzymes involved in the synthesis of glucose and fibers, respectively. The results and the methodological approach described herein can also be consulted in more detail by Hernández-Soto (2019) and Kariñho-Betancourt et al. (2019).

Methodological Approach

Gall Sampling

Galls of *Amphibolips michoacaensis* (Cynipidae) and nongalled tissues were collected from a single tree of *Quercus castanea* (Fagaceae) in central Mexico. According to the histological study of Hernandez-Soto et al. (2015) described in Box 17.3, seven galls (7, 8, 11, 13, 18, 25 and 52 mm) representing different stages of development in the growth phase (hereafter EG, early; IG, intermediate; and LG, late growth stages) and two adjacent undamaged leaves in the early developmental stage (nongalled, NG) were sampled. Nongalled and gall tissues were frozen in liquid nitrogen and stored at −70 °C.

Sequencing and Transcriptome Assembly

Total RNA was extracted from galls and nongalled tissue. Nine TruSeq libraries were prepared (insert size of ±480 bp) and sequenced using the HiSeq 2500 platform in a paired-end 2 × 100 mode. The transcriptome was assembled de novo using Trinity (trinityrnaseq-2.0.6) (Grabnherr et al. 2011) after the quality of the sequences was optimized using FastQC and Trimmomatic command lines (Bolger et al. 2014). The reads of all sequenced libraries were combined, and the longest isoform was extracted from each of the 47,675 components for downstream analysis.

Differential Gene Expression Analysis and Functional Annotation

Read counts per component were estimated with eXpress (expr4.0), and the differential gene expression analyses were performed using the software package EdgeR after filtering out non-Viridiplantae sequences (Robinson et al. 2010). Samples were grouped into three categories using multidimensional scaling (MDS). These three categories corresponded to the developmental stage of galls (early, intermediate, or late), and an additional category corresponded to the nongalled tissue. Based on MDS, the 11 mm sample was not included in the rest of the analyses. Read counts were normalized to counts per million (CPM). Differential gene expression [false discovery rate (FDR) <0.01, $P \leq 0.05$, log2-fold change (FC) ≥ 1] was assessed by comparing galls from the three growth stages and the nongalled tissue. Gall-specific genes were identified as genes showing less than 1CPM in nongalled samples and at least 1CPM in two or more gall samples.

The longest isoforms were subjected to Blast searches against the NCBI nr database (blastx -evalue 1e-5 -num_alignments 5) (Altschul et al. 1997), and the xml output was further annotated with Blast2GO to obtain the corresponding gene ontologies (Götz et al. 2008). GO enrichment analyses were performed with a false discovery rate ≤ 0.01, and based on the results, all differentially expressed transcripts related to development, amylose and phenolic metabolism, were identified.

A heat map was generated from the list of significantly expressed genes linked to phenols in at least one of the gall stages compared by using the heatmap.2 function in the ggplot2 library in R with the scale option by row (R Development Core Team 2014; Wickham 2016).

Differential Gene Expression

The de novo assembly of the transcriptome from galled (G) and nongalled (NG) tissues of *Q. castanea* resulted in 48,705 contigs (longest sequence of each cluster), with a protein prediction of 51.2%, among which 28.66% contigs were associated with a GO term. Gene expression analysis of G vs. NG revealed 11,816 differentially expressed transcripts (Fig. 17.2), of which 2627 were gall-specific.

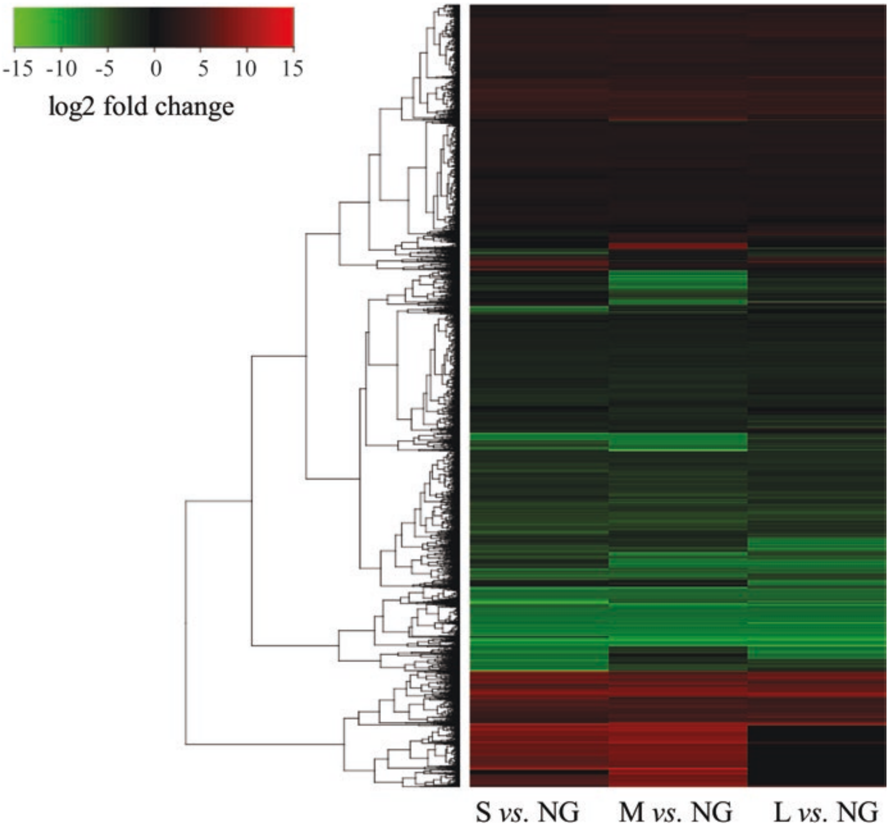


Fig. 17.2 Heat map of differentially expressed genes in galls of *Quercus castanea* during growth phases (early growth, EG; intermediate growth, IG; late growth, LG) and non-damage leaves (NG). (Modified from Kariñho-Betancourt et al. 2019)

Gall Regulation

The functional categories of significantly upregulated genes derived from the G vs. NG comparison were associated with four main processes: (1) cell division, (2) growth and organization of the cell wall, (3) carbohydrate metabolism, and (4) secondary metabolism. Cell division included 14 categories of genes associated with DNA enzymes and cell-division proteins, among others. The growth and organization of the cell wall included six categories of genes related to cell wall biogenesis. Carbohydrate metabolism included five categories related to primary, starch, hexose, sucrose, and polysaccharide metabolic processes, whereas secondary metabolism included six categories mainly related to phenolic biosynthesis and oxidative enzymes. The downregulated genes identified G vs. NG only correspond to categories of growth and organization of the cell wall and secondary metabolism related to oxidative enzymatic functions (laccases and peroxidases) (Table 17.2).

Table 17.2 Functional categories and gene products differentially expressed in *Quercus castanea* galls induced by *Amphibolips michoacaensis*

Functional categories	Gene ontology terms	Upregulated gene products	Downregulated gene products
Cell division			
Cell cycle and DNA replication	0044786	Cyclins	
Regulation of cell cycle	0051726	Cyclin-dependent kinases	
DNA metabolic process	0006259	Cdk-activating kinases	
Purine ribonucleoside binding	0032550	Cell-division cycle proteins	
Nuclear division	0000280	Cyclase-associated proteins	
Unidimensional cell growth	0009826	Mitogen-activated protein kinases	
Chromosome	0005694	Anaphase-promoting protein subunit	
Chromatin assembly	0031497	G2 mitotic-specific cyclin	
Organelle organization	0006996	Mitotic checkpoint protein	
Intracellular membrane-bounded organelle	0043231	Kinetochore protein	
Cytoskeleton	0005856	Chromatin assembly factor 1	
Cytoskeleton organization	0007010	Chromosome-associated kinesin	
Microtubule cytoskeleton organization	0000226	Telomerase reverse transcriptase	

(continued)

Table 17.2 (continued)

Functional categories	Gene ontology terms	Upregulated gene products	Downregulated gene products
Microtubule-associated complex	0005875	Proliferating cell nuclear antigen	
		Replication factor c DNA polymerase	
		DNA polymerases	
		DNA helicases	
		DNA repair proteins	
		DNA topoisomerases	
		DNA replication licensing factors	
		Tubulins	
		Kinesins	
		Microtubule-associated proteins	
Growth and organization of cell wall			
Cell wall biogenesis	0042546	Cellulose synthases	Wall-associated kinases
Cellulose metabolic activity	0030243	Endoglucanase	Wall-associated receptor kinases
Plant-type cell wall biogenesis	0009832	Fasciclin arabinogalactans	Cell wall invertases
Cell wall organization	0071555	Polygalacturonases	Laccases
Plant-type cell wall organization	0009664	Rhamnogalacturonate lyases	Xyloglucan galactosyltransferase
Cell wall thickening	0052386	Pectin lyases	
		Pectate lyases	
		Pectinesterases	
		Pectin methylesterases	
		Expansins	
		Xyloglucan endotransglycosylases	
		Callose synthases	
Carbohydrate metabolism			
Starch metabolic process	0005982	Starch synthases	
		Starch-branching enzymes	
Glucose catabolic process	0006007	Glucose-6-phosphate (G6P)	
Hexose metabolic process	0019318	6-phosphofructo-2-kinase/fructose–bisphosphatases	
Polysaccharide metabolic process	0005976	Amylases	

(continued)

Table 17.2 (continued)

Functional categories	Gene ontology terms	Upregulated gene products	Downregulated gene products
Sucrose metabolic process	0005985	Sucrose phosphatases	
Primary metabolic process	0044238	Galactosidases Glycosyl hydrolases	
Secondary metabolism			
Secondary metabolic process	0009699	Phenylalanine ammonia-lyase	Laccases/peroxidases
Secondary metabolite biosynthetic process	0044550	4-coumaroyl:CoA ligase	4-hydroxy-3-methylbutyl diphosphate synthase
Flavonoid metabolic process	0009812	Chalcone synthase	
Glycosinolate metabolic process	0019757	Chalcone isomerase	
Phenylpropanoid metabolic process	0009698	Flavonone 3-hydroxylase	
Lignin metabolic process	0009808	Flavonoid 3-hydroxylase	
		Cinnamyl alcohol dehydrogenase	
		Caffeoyl CoA 3-O-methyltransferase	
		Laccase/peroxidases	
		NAC domain-containing transcription factor	

Gall-Specific Genes During Development

Significant differential expression of genes was documented during the gall development (Fig. 17.3). Among the three gall stages of the growth phase, 3998 transcripts were differentially expressed. In the IG vs. EG (intermediate vs. early stages) comparison, 1134 genes were upregulated and 1109 were downregulated. In LG vs. IG (late vs. intermediate stages) comparison, 1959 genes were upregulated and 1363 were downregulated. In the two most contrasting developmental phases of galls, LG vs. EG (late vs. early stages), 1975 genes were upregulated and only 1387 were downregulated.

Across the three stages of gall growth, genes associated with functional categories related to development, carbohydrates, and secondary metabolism were differentially expressed. The functional categories of development were related to (1) cell cycle, (2) DNA processes, and (3) morphogenesis, all of which showed downregulation at the IG (intermediate) and LG (late) stages of growth (Table 17.3). The expression patterns of nutrient (starch and soluble sugars)- and defense (phenolic compounds)-related genes are detailed below.

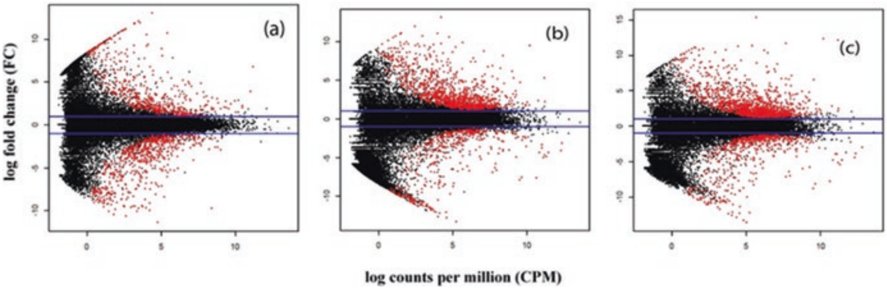


Fig. 17.3 Differential gene expression in the transcriptome obtained from galls of *Quercus castanea* induced by *Amphibolips michoacaensis*, during development. (**a**) Galls at the intermediate vs. early stages of growth (IG vs. EG), (**b**) galls at the late vs. intermediate stages of growth (LG vs. IG), and (**c**) galls at the late vs. early stages of growth (LG vs. EG). (Modified from Kariñho-Betancourt et al. 2019)

Table 17.3 Gene expression and functional categories from *Quercus castanea* transcriptome associated with development, carbohydrate metabolism, and secondary metabolism, during three growth phases: EG, early; IG, intermediate; and LG, late stages. Contrasts between each category are indicated as follows: IE (intermediate vs. early stage), LI (late vs. intermediate stage) and LE (late vs. early stage). Level of gene expression is indicated as UP (upregulated genes) and DOWN (downregulated genes). (Modified from Kariñho-Betancourt et al. 2019)

Expression level	Functional category	Gene ontology (GO-ID)
		Cell cycle
DOWN-IE	Cell division	0051301
	Cell cycle	0007049
	Cytoskeleton-dependent cytokinesis	0061640
	Mitotic cytokinetic process	1902410
	Mitotic cell cycle process	1903047
	Regulation of cell cycle	0051726
DOWN-LE	Cell division	0051301
	Cell cycle	0007049
	Cytokinesis	0000910
	Mitotic cytokinetic process	1902410
	Mitotic cell cycle phase transition	0044772
	Regulation of mitotic cell cycle	0007346
	Regulation of cell cycle	0051726
		DNA process
DOWN-LE	Chromosome organization	0051276
	DNA alkylation	0006305
	DNA modification	0006304
	Methylation	0032259
	Nucleic acid metabolic process	0090304
	Regulation of DNA metabolic process	0051052

(continued)

Table 17.3 (continued)

Expression level	Functional category	Gene ontology (GO-ID)
		Morphogenesis
DOWN-IE	Floral organ formation	0048449
	Organ formation	0048645
Secondary metabolism (Phenolic biosynthesis)		
UP-IE	Flavonoid metabolic process	0009812
UP-LI	Secondary metabolic process	0009699
	Secondary metabolite biosynthetic process	0044710
UP-LE	Secondary metabolite biosynthetic process	0044550
	Secondary metabolic process	0019748
	S-glycoside biosynthetic process	0016144
	Glycosinolate metabolic process	0019757
	Phenylpropanoid metabolic process	0009698
	Secondary metabolic process	0006558
DOWN-LI	Flavonoid metabolic process	0009812
	Lignin metabolic process	0006950
DOWN-LE	Lignin metabolic process	0009808
	Phenylpropanoid metabolic process	0009698
	Single organism process	0044699
	Secondary metabolic process	0019748
		Starch metabolism
DOWN-LE	External encapsulating structure Cell part Cellular component organization or biogenesis Cellular process	0030312 0044464 0071840 0009987
		Hydrolase activity
UP-IE	Organic substance metabolic process macromolecule metabolic process	0071704 0043170
UP-LE	Organic substance metabolic process macromolecule metabolic process	0071704 0043170
		Oxidative activity
UP-IE	Oxidoreductase activity	0016491
UP-LI	Laccase-17-like isoform x2	0046914
	Peroxidase superfamily protein	0006950
	Peroxidase 10	0044710
UP-LE	Oxidoreductase activity	0016491
DOWN-LI	Peroxidase superfamily protein	0044710
	Putative laccase-9-like	0005576

Modified from Kariñho-Betancourt et al. (2019)

Table 17.4 Differential gene expression of *Quercus castanea* galls induced by *Amphibolips michoacaensis.Gene function associated with nutrient-related enzymes at the early (GI), intermediate (GI) and late (GL) stages of growth.*

Galls at the intermediate vs. *early growth stage* (IG vs. EG)		
	Upregulated	**Downregulate**
Enzyme/protein	**Gen function (Annotation)**	
Hydrolase related	(1) Glycosyl hydrolases family protein **(GHase)**	
Galls at the late vs. *early growth stage* (LG vs. EG)		
	Upregulated	**Downregulate**
Amylase related		(1) Glucose-6-phosphate **(G6P)**
Hydrolase related	(1) Glycosyl hydrolases family protein **(GHase)**	

The contrast of galls at the late vs. intermediate growth did not involve starch-related genes

Nutrient-Related Genes

Amylose-related genes were downregulated at the late stage of growth. Amylose genes were only related to protein glucose-6-phosphate (G6P) (Table 17.4). Genes related to hydrolase activity were upregulated at the intermediate (IG) and late (LG) stages of growth. The hydrolase family genes comprise the glycosyl hydrolases (GHase), which are involved in the metabolism of carbohydrates, and some of these genes such as galactosidases might be involved in nutrient enrichment.

Defense-Related Genes

Functional categories of secondary metabolism were related to phenolic biosynthesis (flavonoid and lignin metabolic processes) and oxidoreductase and catalytic activities (Table 17.3). Genes related to phenylpropanoids (e.g., flavonoids) showed an increase in expression at the early (EG) and intermediate growth stages (IG), whereas genes that were mostly related to lignin biosynthesis exhibited increased expression at the late developmental stage (LG) (Fig. 17.4). Nevertheless, only the upregulation patterns of phenylpropanoids were significant [log fold-change (FC) >1]. Genes associated with the enzyme phenylalanine ammonia-lyase (PAL) were significantly upregulated at both intermediate (IG) and late (GL) growth stages. During (IG), genes related to specific enzymes of the phenylpropanoid cascade such as the chalcone synthase (CHS) and flavanone 3-hydroxylase (F3H) were

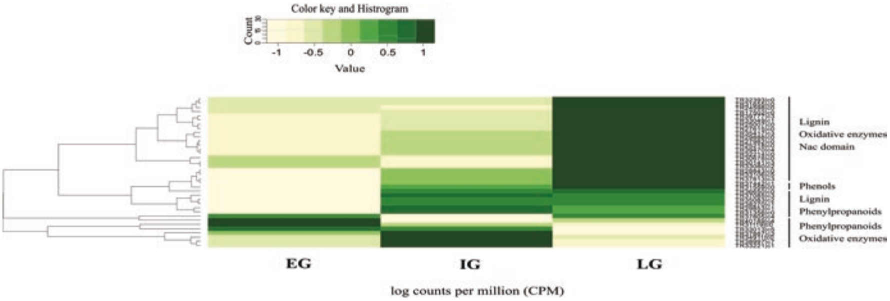


Fig. 17.4 Heat map of differentially expressed phenolic-related genes of *Quercus castanea* during growth phases. Expression values of all genes that were differentially expressed (log2 fold change ≥1, p-value <0.05, FDR <0.01) in at least one contrast are presented as CPM normalized log2 transformed counts. Beige and dark green colors indicate down- and upregulated transcripts, respectively. Each row represents a gene related to main phenolic biosynthetic routes (lignin and phenylpropanoid routes). Columns represent each category of gall growth. *EG*, early stage; *IG*, intermediate stage; and *LG*, late stage of growth. (Modified from Kariñho-Betancourt et al. 2019)

upregulated. Additionally, genes related to oxidative enzymes such as peroxidases and laccases (PER/LAC) showed an increase in expression (Table 17.5). In (LG), genes associated with lignin enzymes and transcriptional factors of the NAC domain were upregulated (Fig. 17.4).

Genes associated with flavonoids, oxidoreductase activity, and lignin showed reduced expression at different growth stages (Fig. 17.4). However, only genes related to phenylpropanoid enzymes such as chalcone synthase (CHS) and flavonoid 3’hydroxylase (F3’H), along with oxidative enzymes, were significantly downregulated at the late growth stage (LG).

Significance of Gene Expression Patterns

The main processes associated with the patterns of differential gene expression observed in galls of *Q. castanea* were cell cycle, cell wall biogenesis and organization, carbohydrate metabolism, and secondary metabolism related to phenolic compounds. Overall, most of the differentially expressed genes showed increased expression in galled tissues. Genes related to the cell cycle were only upregulated in galls, only a few genes related to enzymatic activity were involved in the synthesis of the cell wall, and phenolic compounds were up- and downregulated in both galled and ungalled tissue. These results show that the genetic control of galls is very different from that of ungalled leaf tissue. We discuss the main patterns of gene expression related to development, nutrients, and defense.

Table 17.5 Differential gene expression of *Quercus castanea* galls induced by *Amphibolips michoacaensis. Gene function associated with defense-related enzymes at the early (GI), intermediate (GI) and late (GL) stages of growth. (Modified from Kariñho-Betancourt et al.* 2019)

Galls at the intermediate vs. *early growth stage* (IG vs. EG)		
	Upregulated	**Downregulated**
Enzyme/protein	**Gen function (Annotation)**	
Common for phenols	(1) Phenylalanine ammonia-lyase **(PAL)** (2) 4-coumaroyl:CoA ligase **(4CL)**	
Common for phenylpropanoids	(1) Chalcone synthase **(CHS)** (2) Chalcone isomerase **(CHI)** (3) Flavanone 3-hydroxylase **(F3H)** (4) Flavonoid 3´hydroxylase **(F3´H)**	
Galls at the late vs. *early growth stage* (LG vs. IG)		
	Upregulated	**Downregulate**
Common for phenols	(1) Phenylalanine ammonia-lyase **(PAL)**	
Common for phenylpropanoids		(1) Chalcone synthase **(CHS)** (2) Chalcone isomerase **(CHI)** (3) Flavonoid 3´hydroxylase **(F3´H)**
Common for lignin	(1) Cinnamyl alcohol dehydrogenase **(CAD)** (2) Caffeoyl CoA 3-O-methyltransferase **(CCoAOMIT)** (3) Peroxidase/laccase family **(PER/LAC)**	
Galls at the late vs. *early growth stage* (LG vs. EG)		
	Upregulated	**Downregulate**
Common for phenols	(1) Phenylalanine ammonia-lyase **(PAL)**	
Common for phenylpropanoids	(1) Flavonoid 3´hydroxylase **(F3´H)**	
Common for lignin	(1) Caffeoyl CoA 3-O-methyltransferase **(CCoAOMIT)** (2) Peroxidase/laccase family **(PER/LAC)**	(1) Peroxidase/laccase family **(PER/LAC)**
Transcriptional factors	(1) NAC domain	

SRA accession number: PRJNA532454

Growth and Developmental Processes

Our findings indicate that the expression of genes linked to the processes of differentiation and cell growth increases in galls. This suggests that gene regulation is strongly involved in the development of the induced abnormal plant growth. In addition, across the developmental stages of the gall growth phase, we found that genes related to the cell cycle, growth, and organization of the cell wall and morphogenesis were downregulated at the intermediate (IG) and late (LG) stages of growth, suggesting increased expression in the early stage of growth (EG). This evidence is consistent with cell proliferation or hyperplasia documented in histological analyses during the early stages of gall growth induced by cynipid wasps across diverse plant taxa and among different plant tissues (Jansen-González et al. 2014; Reale et al. 2016). Hence, the patterns of gene expression linked to the cell cycle in cynipid galls suggest that gall development is closely tied to the insect's growth, which accelerates during the early developmental stage and slows during late growth.

Nutrient-Related Patterns

Starch serves as the major carbohydrate reserve in specialized plant cells (Zeeman et al. 2010). Each starch granule contains several million amylopectin molecules accompanied by a much larger number of smaller amylose molecules. Amylose is composed of alpha-D glucose units, which are the substrate for the isomerase D-glucose-6-phosphate 1-epimerase during glycolysis (Tetlow et al. 2004; Martin and Smith 1995). In our study, when comparing the late vs. the early stage of growth (LG vs. EG), we found that genes related to carbohydrate synthesis, such as those linked to the enzyme D-glucose-6-phosphate (G6P), were downregulated at the late stage (LG), suggesting an increased expression in the early stage of growth. These results are consistent with the histological study of galls induced by *A. michoacaensis* in *Q. castanea*, which showed that amyloplasts increase during early growth and begin to degrade once the gall reaches maturity (Hernández-Soto et al. 2015). Amyloplast-filled cells constitute the main food source for growing larvae (Guzicka et al. 2017), because these cells are responsible for the synthesis and storage of starch granules through the polymerization of glucose (Wise 2007). The depletion of amyloplasts during the development of cynipid galls may reflect the adjustment of the nutritional needs of the larvae as they approach the pupa stage. The histological and genetic evidence from the cynipid galls of *Q. castanea* indicates that starch-related patterns play a central role in the oak-cynipid interaction.

Defense-Related Patterns

Oaks (*Quercus* spp.) are characterized by the production of several defense-related phenolic compounds, including phenylpropanoids (e.g., flavonoids and coumarins) and lignin (Feeny 1976; Forkner et al. 2004; Pearse and Hipp 2012; Moctezuma et al. 2014). Phenolics can alter the enzymatic digestive activity of herbivores, exerting a toxic and/or antifeedant effect (Barbehenn and Constabel 2011). Many of these compounds occur in galls induced by cynipid wasps on oaks. When comparing the cynipid galls of *Q. castanea* with ungalled tissues, we found that genes related to phenolic biosynthesis showed increased expression in the galled tissues, suggesting that the concentration of phenolics is higher in galls. The increased expression of genes linked to phenolic enzymes is correlated with the accumulation of phenolic compounds (e.g., Howles et al. 1996; Muir et al. 2001). Hence, our results agree with the increases in phenolic concentration in gall tissues reported in several oak species (Hartley 1998). The accumulation of phenolic compounds in galls has been implicated in defense mechanisms against parasitoids, gallivores, generalist folivores, or fungal infection (Cornell 1983; Schultz 1992; Pascual-Alvarado 2008). In oak galls, specific compounds show a positive effect on galling insects. For instance, Taper and Case (1987) found a positive effect of leaf tannin levels in oak galls on the number of species of leaf-galling cynipid wasp present. This evidence suggests a protective role of phenolics against parasitic wasp attack.

In addition, we found that the regulation of phenolic genes in galls varies across development. In the galls of *Q. castanea* phenylpropanoid-related genes were upregulated at the intermediate stage (the peak of growth) and downregulated at the late growth stage (LG) when galls are close to mature, and the process of lignification is in progress. In contrast, lignin-related genes were upregulated at the late stage of the growth phase. The PAL enzyme was upregulated at both the intermediate and late growth stages. This enzyme triggers the first step in the biosynthesis of phenols, fueling the various routes by which different kinds of phenolic compounds are synthesized, including phenylpropanoids and lignin (Hahlbrock and Scheel 1989). The upregulation of PAL along with enzymes related to the phenylpropanoid branch during the peak of gall growth suggests that this developmental phase is the most active in the synthesis of defensive compounds, such as flavonoids, and is probably the developmental phase in which the insect is most vulnerable and requires an increase in chemical defenses. On the other hand, genes associated with complex structural organic polymers such as lignin and specific transcriptional factors of the NAC domain family and oxidative enzymes showed increased expression with gall age. Lignin plays a central role in the formation of cell walls because it provides rigidity and support. In addition, the NAC domain and oxidative enzymes have been strongly implicated in the regulation of secondary wall synthesis in fibers (Zhong et al. 2006). These results are consistent with the developmental trajectory documented in the histological study of *Q. castanea* galls induced by *A. michoacaensis* (i.e., an increase in lignified cells with gall age) (Hernández-Soto et al. 2015).

In addition to the adaptive meaning of the contrasting metabolic dynamics between lignin and phenylpropanoid-related genes found in wasp galls, this pattern may indicate constraints via channeling the phenolic metabolic flux. Substantial evidence indicates that enzyme complexes involved in phenylpropanoid metabolism antagonize lignin metabolic pathways (Stafford 1990; Koes et al. 1994; Shirley 1996; Winkel-Shirley 1999). Empirical evidence has shown that flavonoid metabolism competes directly with pathways leading to lignin and its precursors (e.g., hydroxycinnamic compounds) (Howles et al. 1996). Accordingly, our results suggest that lignin biosynthesis may occur at the expense of that of phenylpropanoids within the sampled tissues of oak galls induced by *A. michoacaensis*.

Conclusions and Future Directions

The transcriptomic profiles of *Q. castanea* galls induced by *A. michoacaensis* reflect the changes in the cell cycle and metabolism during gall development, highlighting the importance of enzymes involved in starch and phenol biosynthesis in the ecological dynamics of the plant-insect interaction. The differential expression patterns observed in wasp galls suggest phenotype manipulation by the wasp larvae and physiological constraints of the host plant for enzyme channeling in different biosynthetic branches of phenolic compounds.

This study is a good example of how analytic methods based on high-throughput sequencing to explore gene function in natural populations of nonmodel species can elucidate the way in which an organism interprets signals to activate developmental programs or responds to environmental stimuli, thus contributing to revealing the mechanistic bases of the interactions between organisms. Nevertheless, an important challenge in this type of study is that different cells can show different patterns of gene expression, so new third-generation massive sequencing technologies (e.g., NovaSeq) as well as the development of equipment that allow the dissection of cell types (e.g., laser dissection microscopes) or the use of the 10X Genomics platform to carry out single cell transcriptomics will make it possible to analyze gene expression at a finer level in the short term.

Natural variation provides opportunities to address fundamental ecological and evolutionary questions that cannot be fully answered using model plants alone or by using a single analytical approach. By combining bioinformatics and biostatistics tools and molecular biology, evolutionary, and ecological approaches, the ecological genomics field is developing rapidly. However, the study of the adaptive mechanisms that have evolved in response to biotic stress will continue to be a challenge. Broader collaboration between ecologists, evolutionary biologists and molecular geneticists is crucial to characterize the natural variation within and among species and to elucidate the genetic bases of adaptive traits. To achieve this goal, we suggest that future research in nonmodel plants should consider the classical approach of applied ecology as both the starting and ending points of investigation to characterize ecologically important traits and confirm the functions of genes. The characterization of ecological traits should include experimental and field studies linked to biochemical analyses. This approach may also include the identification of DNA polymorphisms and candidate genes (e.g., quantitative trait locus (QTL) mapping, genome-wide association studies) as well as the study of the phenotypic variation of putative adaptive traits. Hence, this research avenue will contribute to integrating diverse genomic/transcriptomic/proteomic data (e.g., sequences, map positions, mRNA, and protein expression and allelic variation) into an ecological and evolutionary framework.

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Chapter 18
Latitudinal and Elevational Gradients in Plant Defences and Herbivory in Temperate Trees: Recent Findings, Underlying Drivers, and the Use of Genomic Tools for Uncovering Clinal Evolution

Diego Carmona, Xoaquín Moreira, and Luis Abdala-Roberts

Abstract Environmental gradients serve as powerful settings to elucidate the ecological and evolutionary processes driving changes in species diversity, trait evolution, and ecosystem function. Classic theory holds that stronger plant-herbivore interactions under more stable and warmer climates towards the equator and sea level have resulted in stronger selection on plant defences. We hereby address latitudinal and elevational gradients in plant defences and herbivory follow these predictions for a number of dominant taxa of temperate trees. Many of these taxa include species that span broad latitudinal and elevational ranges and thus represent useful models for testing clinal variation in plant defences and herbivory. First, we review recent studies testing for latitudinal and elevational gradients in temperate tree defences and herbivory. Second, we analyse these results in the light of classical theory and discuss potential deviations from expected patterns and candidate mechanisms. Third, we analysed the use of genomic tools for assessing the genetic basis of clinal evolution in plant defences, a promising alternative toward reducing inconsistencies and identifying commonalities in ecological and evolutionary processes. Our review indicates considerable variation in the strength and direction of elevational and latitudinal gradients in temperate tree defences and herbivory. Strikingly, patterns that are opposite to classic predictions are equally common and, in some cases, even more common than expected patterns. In light of these findings, we argue for a need to apply consistent methods across studies, conduct more comprehensive assessments of plant defensive phenotypes, and explicitly consider the role of abiotic factors. Furthermore, as future research closes these gaps, the adoption of genomic tools will open an unprecedented opportunity to launch a new generation of studies.

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To achieve this, there is a need to merge research on landscape genetics and ecological studies of plant-intraspecific clines in plant-herbivore interactions to unveil the genetic basis of clinal evolution in plant defences. Likewise, analyses of the molecular level evolution of target genes associated with plant defence also hold a large potential for assessing plant defence macro-evolutionary patterns along environmental clines. Applying these tools will help elucidate the mechanisms of adaptive evolution in plant defence along environmental clines and contribute to develop new theory by uncovering patterns not apparent previously from studies based solely on measurements of plant phenotypes and species interactions.

Keywords Clinal variation · Elevational gradients · Environmental gradients · Evolutionary genomics · Latitudinal gradients · Plant defence · Macroevolution

Environmental gradients have served as powerful settings to elucidate the ecological and evolutionary processes driving changes in species diversity, trait evolution, and ecosystem function (Dobzhansky 1950; Pianka 1966; Reich and Oleksyn 2004; Schemske et al. 2009). Latitudinal and elevational clines in biodiversity and species interactions have been especially well studied in this regard (Schemske et al. 2009; Sundqvist et al. 2013; Anstett et al. 2016), and although they differ in some aspects (see Moreira et al. 2018a), they are united under a common paradigm underscoring the role of abiotic controls over species abundances, diversity, and interaction strength. Under this framework, species interactions are predicted to be more intense under less seasonal and warmer climates found at lower latitudes and elevations, and this results in stronger selection on species traits (Schemske et al. 2009; Moreira et al. 2018a; Rasmann et al. 2014a; Mittlebach et al. 2007). In the case of plant-herbivore interactions, more intense and consistent patterns of herbivory under more stable and warmer climates toward the equator and sea level should result in stronger selection on plant defences (Rasmann et al. 2014a; Baskett and Schemske 2018). In turn, this is thought to have promoted plant (herbivory-based) niche differentiation, coexistence and speciation (Schemske et al. 2009; Kursar et al. 2009; Coley and Kursar 2014), and, indirectly, herbivore diversification (Futuyma and Agrawal 2009).

The mechanisms driving elevational and latitudinal variation in plant-herbivore interactions have been intensely debated, and a general consensus is yet to be reached (Anstett et al. 2016; Moreira et al. 2018a). The main reason has been the lack of consistency in the strength and direction of quantitative or qualitative variation in plant defences with latitude (Anstett et al. 2016; Moles et al. 2011) and elevation (Moreira et al. 2018a; Rasmann et al. 2014b). For example, Moles et al. (2011) found that merely 16% of studies conducted up to that date had found evidence that plants at lower latitudes were more defended than high-latitude plants, and a recent review by Moreira et al. (2018a) indicated that 66% of studies found that plants at low elevations were more highly defended than their high-elevation counterparts. A series of explanations have been put forward to account for these inconsistent patterns, ranging from contrasting spatial and taxonomical scales of analyses across studies and insufficient quantification of different types of defensive traits and strategies to a lack of consideration of plant life histories and methodological inconsistencies (Anstett et al. 2016; Moreira et al. 2018a; Kooyers et al. 2017). Moreover, even though the influence of abiotic factors on herbivory is implicit in most research, only until recently studies began to explicitly address the influence of abiotic correlates of latitude and elevation (e.g. climate, soil variables) on plant defences and herbivory (Moreira et al. 2018a; Johnson and Rasmann 2011). Therefore, it has been difficult to identify the underlying factors dictating these disparate patterns (but see Galmán et al. 2018), and this has limited our understanding of the generative processes by which abiotic clines shape plant defences and herbivore pressure.

Another important consideration is that the literature on latitudinal and elevational gradients in plant-herbivore interactions has centred more frequently on herbaceous plants or shrubs, while studies performed on tree species have been relatively less common. Nonetheless, over 30% of the Earth's land surface is covered by forests (FAO 2012), and tree species account by and large most of the biomass in forested ecosystems (Grossman et al. 2018). In temperate regions, for example, there are a number of dominant tree taxa that cover large expanses of land and play preponderant role in shaping species interactions, evolutionary dynamics, and ecosystem function (e.g. *Quercus*, *Pinus*, *Betula*, *Salix*, among others). In addition, many of these temperate tree species span broad latitudinal and elevational ranges (e.g. *Pinus* (Moreira et al. 2014), *Quercus* (Galmán et al. 2019)) and therefore represent highly suitable models to test for clinal variation in plant defences and herbivore pressure. Addressing these patterns and their underlying drivers for temperate tree species is therefore needed to increase inference and achieve a more robust understanding of the evolutionary ecology of plant-herbivore interactions under a spatial context.

Crucially, research on elevational and latitudinal gradients in plant defences and herbivory has a deeply rooted evolutionary perspective (Dobzhansky 1950; Schemske et al. 2009; Endler 1977). Paradoxically, clinal evolution of species traits is often implied in these studies but relatively few have involved explicit evaluations of evolutionary processes. The most notable exception to this is classical (and a number of more recent) studies involving common garden and reciprocal transplant experiments to test for population differentiation along (e.g. elevational) clines (reviewed by Cheplick 2015). Still, much of this research has focused largely on plant traits associated with adaptation to abiotic factors (Clausen et al. 1947; Linhart and Grant 1996), and the fewer studies addressing clinal adaptation to biotic factors have mostly focused on traits associated with competition and to some extent also pollination (Cheplick 2015). In contrast, tests of local adaptation to herbivores and pathogens are far less common (Abdala-Roberts and Marquis 2007; Lowry et al. 2019), and this bias is also markedly reflected in research on latitudinal and elevational gradients. As a result, very few studies have involved direct and explicit evaluations of the evolution of plant defences along elevational and latitudinal clines (but see Pellissier et al. 2014). Generally speaking, a number of analytical tools have flourished over the last decade which can be used to detect loci (e.g. defensive genes) under selection both within and across plant taxa. These range from genome-wide association analyses within species (e.g. De Kort et al. 2014) to molecular analyses pinpointing macro-evolutionary patterns of selection on plant defences across plant taxa (Carmona et al. 2019). Still, many of these methods have rarely been applied to plant genes associated with biotic defence under the geographic context of latitudinal and elevational gradients, thus pointing at a highly promising but yet unrealized research opportunity.

In this chapter, we focus on the evolutionary ecology of latitudinal and elevational gradients in plant defences and herbivory associated with long-lived temperate trees, though the arguments and conclusions drawn from our review in general terms extend to plant species of any life form. First, we review a suite of studies conducted over the last decade testing for latitudinal and elevational gradients in defences and herbivory for several genera of temperate trees which have served as model systems. Second, we compare these results to classical predictions on latitudinal and elevational gradients in herbivore pressure and discuss the observed patterns. Here we also include an explicit consideration of abiotic controls over clinal variation in plant traits and herbivory, which a few recent studies have addressed. Third, we discuss classic and modern approaches that have been used to study the evolutionary implications of clinal variation in plant defences. Here we emphasize the potential application of genomic tools to measure and understand adaptive evolution of plant defences along ecological gradients, and provide examples of ways in which they can be used to address questions at both the micro- and macro-evolutionary level.

Latitudinal and Elevational Gradients in Plant-Herbivore Interactions in Temperate Trees

We next review the main findings from studies on latitudinal and elevational gradients in plant defences and herbivory for several key genera of temperate trees, mainly oaks (*Quercus* spp.) for which much of the recent work has focused, and to some extent also other groups such as birches (*Betula* spp.), pines (*Pinus* spp.), and beeches (*Fagus* spp.). We focus on these taxa for two reasons. First, they lend themselves to robust tests of intraspecific clines in plant defences and herbivory and their underlying micro-evolutionary processes because they include a number of dominant species spanning broad latitudinal and elevational ranges. For example, *Quercus* and *Pinus* span both tropical and temperate latitudes and up to 3000 m in elevation, covering a vast diversity of abiotic (e.g. climatic) and biotic conditions (Moreira et al. 2014; Cavender-Bares 2019). Second, they provide useful models for assessing macro-ecological and macro-evolutionary patterns of clinal variation because their constituent species vary substantially in their latitudinal and elevational distributions (i.e. large cross-species variation along both geographical axes increasing power of analyses). In addition, working with groups of congeneric species allows for tight controls of ecological variation and phylogenetic relatedness among taxa.

Latitudinal and Elevational Variation in Defences in Temperate Trees

A total of 15 studies spanning 97 species have assessed latitudinal and elevational gradients in defences in temperate trees, of which 80% have involved chemical defences (Fig. 18.1a). Of this total, oaks (*Quercus* spp.) have been the most frequently studied group (53% of studies, 63 species), followed by pines (*Pinus* spp., 33% of studies, 20 species) and birches (*Betula* spp., 13% of studies, three species). A number of these studies have found support for the classic prediction that plants at low latitudes and elevations have higher levels of defences in response to more intense herbivore pressure (Anstett et al. 2016; Rasmann et al. 2014a), namely, 33% of latitudinal studies and 40% of elevational studies. Nonetheless, a moderate amount of studies have found either no evidence of latitudinal (17%) or elevational (20%) clines in defences, and a substantial number of other studies have found, contrary to expectations, greater levels of defences at higher latitudes (50%) or elevations (40%).

Variation among studies in the strength and direction of latitudinal and elevational gradients in plant defences could be explained by differences in the type and identity of plant defensive correlates measured as well as the resolution of chemical analyses (Anstett et al. 2016; Moreira et al. 2018a). For example, the identity of specific types of secondary compounds and their role in herbivore resistance is often not assessed as studies typically bin multiple types of compounds into broad classes which may mask clines that are apparent only when looking at individual compounds or groups of compounds (Anstett et al. 2014, 2015). Recent studies with temperate trees that have measured multiple types of secondary compounds (5 studies, 33% of total reported above) highlight this point, as clinal patterns often vary among compound groups or individual compounds (e.g. phenolics, terpenes). Likewise, the functional role of these compounds in determining herbivore resistance for a given plant species or group of related species is often not assessed but rather assumed based on correlations or evidence from other systems without actual documentation of effects on herbivore performance (Anstett et al. 2016).

In addition, plant macronutrients can also predict herbivory (Mattson 1980; Agrawal 2007) and exhibit latitudinal and elevational gradients (e.g. Reich and Oleksyn 2004). Two recent studies with temperate trees reported that the concentration of leaf phosphorus and nitrogen (two limiting macronutrients for herbivores) in *Q. robur* trees increased towards higher latitudes (Moreira et al. 2018b) and elevations (Abdala-Roberts et al. 2016a). In both cases, however, nutritional traits were not associated with leaf damage suggesting they did not influence clines in herbivory. In addition, Garibaldi et al. (2011a) observed that foliar phosphorus content in *Nothofagus pumilio* forests of the northern Patagonian Andes (Argentina) increased

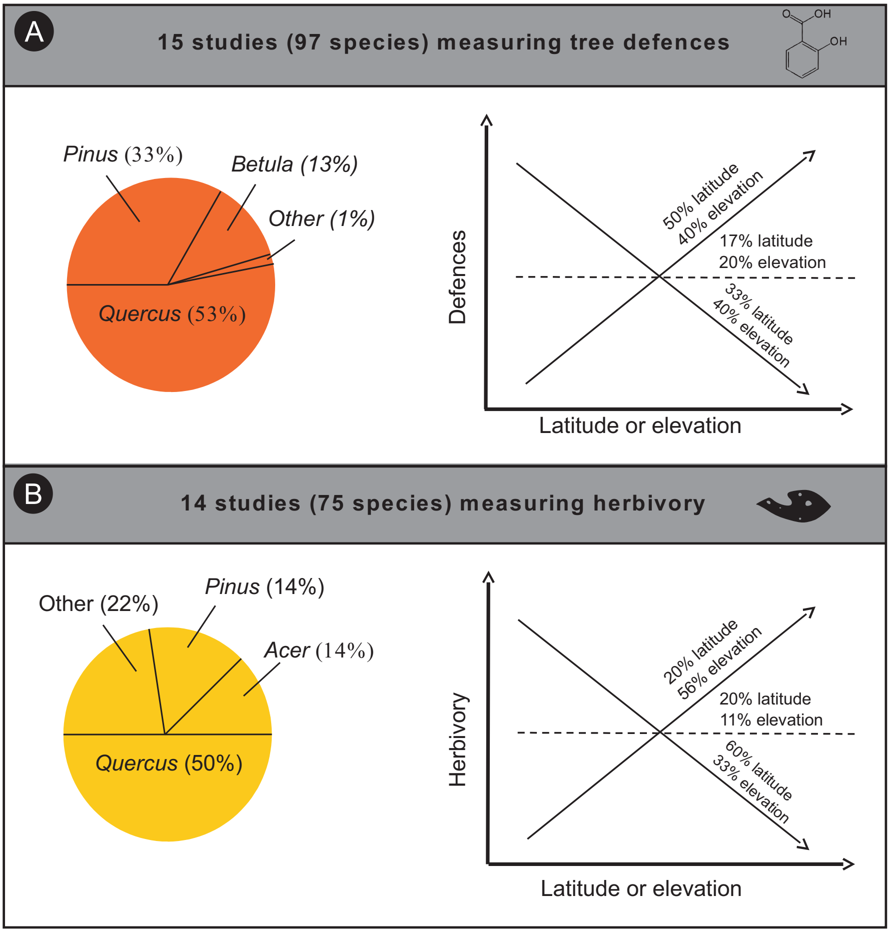

Fig. 18.1 Summary of the number of studies testing for latitudinal or elevational gradients in defences (**a**) and herbivory (**b**) in temperate tree species. The percent of studies showing expected (negative) vs. unexpected (neutral, positive) latitudinal or elevation gradients are shown, as well as percent of studies by tree taxa

from low- to high-elevation sites, but again leaf herbivory and phosphorus concentration were not significantly correlated. Although these studies did not find evidence suggesting that plant nutrients influence clines in herbivory, more studies are still needed. For example, plant exhibit strategies associated with resorption and reallocation of nutritional traits from herbivore-damaged to undamaged tissues (Frost and Hunter 2008; Moreira et al. 2012), and these mechanisms could be under selection by herbivores and vary along ecological gradients of herbivore pressure (or abiotic conditions; see ahead). Therefore, a detailed quantification of both secondary metabolites and nutrients in plant tissues is necessary to better characterize gradients in defence and, more broadly, overall plant quality for herbivores (Marquis et al. 2012) to herbivores along ecological gradients.

A more comprehensive assessment of traits associated with different defensive strategies may also increase our understanding of latitudinal and elevational gradients in plant defence and explain variation in the observed patterns. Defensive strategies such as tolerance (e.g. regrowth capacity or overcompensation in reproduction (Carmona et al. 2011)), induced defences (Karban 2011), and indirect defences (i.e. used to recruit predators and parasitoids (Agrawal 2011)) may also exhibit clinal variation. There may be trade-offs between defensive strategies (Agrawal 2000), such that two or more defensive traits or strategies may negatively covary along latitudinal or elevational gradients such that considering them separately may lead to an erroneous or incomplete understanding of observed patterns. For example, constitutive and induced defences frequently trade off (Moreira et al. 2014; Koricheva et al. 2004; Kempel et al. 2011; Rasmann and Agrawal 2011; Rasmann et al. 2015), and shifts in the relative allocation to each strategy may occur along an ecological gradient. Plants growing in resource-poor and stressful environments found at high latitudes or elevations should be selected for increased allocation to constitutive defences, as the cost of replacing tissues consumed by herbivores is higher under low resource conditions (Coley et al. 1985; Endara and Coley 2011). For example, Moreira et al. (2014) found that levels of constitutive resin in 18 pine species increased at higher latitudes and elevations, whereas the inducibility of these traits increased toward the equator. It is also possible, however, that environments with low herbivore pressure typically found at high latitudes or elevations should select for low constitutive and high induced defences because the costs of continuously producing the former are high when damage is low. Supporting this alternative, Galmán et al. (2019) recently found that oak species with high elevational ranges exhibited a greater inducibility of phenolic compounds (hydrolysable tannins).

More studies differentiating constitutive and induced defences are needed in temperate trees (and other life forms as well) to assess these proposed mechanisms for ecological gradients in plant defensive strategies. In addition, we are unaware of studies testing for latitudinal or elevational clines in direct and indirect defences in trees (see Rasmann et al. 2014c; Pellissier et al. 2016 for examples with herbaceous species), representing another overlooked axis of plant defensive strategies. Similarly, trade-offs between tolerance and resistance (Fineblum and Rausher 1995; Agrawal et al. 1999) may lead to covariation in these defensive strategies along ecological gradients. Only two studies to date have tested for and not found trade-offs in these two plant strategies along latitudinal (Więski and Pennings 2014) or elevational (Dostálek et al. 2016) clines, and both involved herbaceous species. We are aware of no clinal studies on tolerance and resistance with long-lived plants, particularly temperate trees.

Latitudinal and Elevational Variation in Herbivory in Temperate Trees

A total of 14 studies spanning 75 species have assessed latitudinal and elevational gradients in herbivory for temperate trees, all of which have involved leaf damage by insects (Fig. 18.1b). Of this total, again oaks (*Quercus* spp.) by far have been the most frequently studied group (50% of studies, 57 species), followed by pines (*Pinus* spp. 14% of studies, three species) and maples (*Acer* spp., 14% of studies, two species). Several of these papers have found support for the prediction that plants at low latitudes and elevations have higher herbivore pressure, namely, 60% of latitudinal studies and 33% of elevational studies. As for plant defences, however, a substantial number of studies have found either no evidence of latitudinal (20%) or elevational (11%) clines in herbivory, and a number of other studies have found, contrary to expectations, greater levels of herbivory at higher latitudes (20%) and elevations (56%). Taken together, these results indicate considerable deviations in the predicted patterns of elevational and latitudinal variation in herbivore attack, which would presumably lead to concomitant variation in clinal patterns for plant defences.

Plausible explanation for the observed variation in patterns of herbivory (and, as a result, plant defences), common to all plant type studies (not only trees), is that herbivore pressure is not always adequately or consistently measured (Zvereva and Kozlov 2019), and the identity of the attacking herbivores is frequently not assessed (Anstett et al. 2016; Moreira et al. 2018a). Herbivore species or guilds may vary in their susceptibility to changing abiotic conditions and may therefore exhibit different (in some cases contrasting) patterns of abundance and damage on focal host plants along environmental clines (Rasmann et al. 2014b; Anstett et al. 2014, 2015; Pennings et al. 2009). Consequently, pooling damage by multiple types of herbivores may obscure the detection of significant patterns only apparent when differentiating damage by each attacker (Anstett et al. 2016; Moreira et al. 2018a; Abdala-Roberts et al. 2015). Recent studies with temperate trees measuring multiple attackers (5 studies, 36% of total reported above) highlight this point, as clinal patterns in herbivory or abundance commonly vary among herbivore species or guilds (e.g. chewers, miners, sap-feeders). For example, Andrew and Hughes (2005) found that the amount of herbivory by sap-sucking and mining insects on *Acacia falcata* trees increased toward more temperate latitudes, whereas chewing herbivory increased toward tropical latitudes. Similarly, Nakamura et al. (2014) found that herbivory by chewing, mining, and galling insects in beech (*Fagus crenata*) in Japan exhibited contrasting latitudinal patterns. Insect miner and galler densities were higher at lower latitudes, whereas leaf-chewer damage herbivory was greater at higher latitudes (Nakamura et al. 2014).

These inconsistencies or coarseness in herbivory measurements explained above have likely also obscured our understanding of how clines in herbivore pressure shape concomitant clines in plant defences. A number of studies have demonstrated that herbivore species from different guilds can exert selective effects on different plant traits or select on the same trait but in a different manner (e.g. Juenger and Bergelson 1998; Carmona and Fornoni 2013; Wise and Rausher 2013). It is therefore plausible for variable or opposing selection by multiple herbivores to lead to unexpected patterns in the strength and direction of latitudinal (Anstett et al. 2014; Moreira et al. 2015; Abdala-Roberts et al. 2016b) and elevational (Descombes et al. 2017; Pellissier et al. 2012) gradients in plant defences. Relatedly, recent work analysing community-level variation in herbivore traits has shown that insect herbivore diet breadth increases with latitude (Forister et al. 2015; Salazar and Marquis 2012) and elevation (Rasmann et al. 2014a; Pellissier et al. 2012). This could result in latitudinal and elevational changes in herbivore selection on plant traits given that specialist herbivores might select for different plant defensive traits or strategies than generalists (Ali and Agrawal 2012). However, these studies have been restricted to herbaceous plants and virtually nothing is known about this for temperate trees.

Abiotic Controls over Plant Defences and Herbivory Along Latitudinal and Elevational Clines

Abiotic conditions represent a third type of factor which may modulate plant-herbivore interactions along environmental gradients. Although the influence of abiotic factors is implicit in research on latitudinal and elevational gradients in plant-herbivore interactions, paradoxically, relatively few studies have explicitly addressed their influence (e.g. climate, soil nutrients (Johnson and Rasmann 2011; Moreira et al. 2018b)). The implicit assumption has been that abiotic conditions (primarily associated with climate) affect herbivore population sizes and feeding season length, and this then shapes herbivore pressure and selection intensity on plant defences (Anstett et al. 2016). However, recent studies with temperate trees assessing the influence of abiotic correlates (e.g. Galmán et al. 2018; Abdala-Roberts et al. 2016a; Garibaldi et al. 2011a; Loughnan and Williams 2019) suggests that this situation is more complex than previously envisioned and that there may be alternative scenarios. For example, Abdala-Roberts et al. (2016a) reported that climatic factors shaped elevational gradients in insect herbivory and chemical defences in *Q. robur*, but such effects were independent of each other (i.e. climate effects on one had no implications on the other). On the other hand, Garibaldi et al. (2011b) found that insect leaf damage in *N. pumilio* decreased with latitude and such gradient was not attributable to climatic effects on plants and herbivores. In addition, also with *Q. robur*, Moreira et al. (2018b) reported that, rather than abiotic factors shaping latitudinal variation in herbivory and in turn defences in this oak species, climate and soil variables influenced plant chemical defences and such effects indirectly shaped the latitudinal gradient in herbivory. Finally, some studies have simply found no influence of latitudinal variation in abiotic factors (e.g. climate) on concomitant patterns of insect herbivory (Moreira et al. 2018b) or defences (Galmán et al. 2019) in temperate trees. For example, in a greenhouse experiment using 1-year-old plants from 18 oak species, Galmán et al. (2019) reported that oak species growing at higher elevations exhibited a greater inducibility of phenolic compounds, but climatic factors (average climatic niches) did not explain this pattern.

Interestingly, recent work by Mikhail Kozlov and collaborators (2013, 2015) across multiple years elucidated interactions between climatic factors and latitudinal clines in herbivory by different insect guilds for birch trees (*Betula pendula* and *B. pubescens*). For example, the abundance of leaf miners in these two birch species significantly decreased with latitude during cold years but was independent of latitude during warm years (Kozlov et al. 2013). However, the load or density of sap-feeders in these two birch species decreased with increasing latitude in typical summers but increased towards the pole in an exceptionally hot summer (Kozlov et al. 2015) (for examples with herbs, see Anstett et al. 2015; Abdala-Roberts et al. 2016b). These studies emphasize the value of addressing abiotic factors, differentiating damage by multiple herbivore guilds, and assessing temporal variation in such associations in order to elucidate the influence of abiotic controls over latitudinal and elevational gradients in plant-herbivore interactions. Although there has been success in using these observational approaches to understand the concurrent effects of abiotic factors—including our own work in oaks (e.g. Moreira et al. 2018b; Abdala-Roberts et al. 2016a)—experimental manipulations of abiotic drivers are also needed to better understand their influence on species interactions and assess causality.

Evolutionary Studies of Latitudinal and Elevational Gradients in Plant Defences

The central tenet of research on latitudinal and elevational gradients is that stronger herbivore pressure towards lower latitudes and elevations has selected for higher plant defences (Schemske et al. 2009; Baskett and Schemske 2018; Rasmann et al. 2014b). Accordingly, most studies to date have measured proxies of plant defences (e.g. chemical and physical) and imply that observed patterns reflect concomitant variation in biotic selective regimes along elevational (reviewed by Moreira et al. 2018a) or latitudinal (reviewed by Schemske et al. (2009) and Mittlebach et al. (2007)) gradients. Although increasing spatial scale and replication along a sampled gradient allow to more robustly ascertain the evolutionary dynamics shaping biogeographical patterns of plant-herbivore interactions (Anstett et al. 2016), to date relatively few studies have used approaches and methods to explicitly measure the evolutionary mechanisms and implications of clinal variation in herbivory (and abiotic factors) for plant defences.

There is a long history of research testing for plant adaptive differentiation along environmental clines (reviewed by Cheplick (2015) and Linhart and Grant (1996)), with a number of early studies focusing on elevational gradients (e.g. Clausen et al. 1940, 1947, reviewed by Cheplick 2015). Several complementary approaches have been used to this end, from analyses that correlate environmental and genetic variation or differentiation (e.g. Gould et al. 2013) to manipulations involving common garden or reciprocal transplant experiments (reviewed by Cheplick 2015 and Hargreaves et al. 2019), and to a lesser extent studies testing differences in the strength of regression-based estimates of phenotypic selection on focal traits along ecological clines (e.g. Etterson 2004; Toju and Sota 2006). To date, however, experimental studies along latitudinal and elevational gradients have focused mostly on plant adaptation to abiotic factors (e.g. climate or soil conditions Cheplick 2015; Pellissier et al. 2014), whereas fewer have addressed the influence of biotic factors. Exceptions to this include work on population differentiation in growth-related traits associated with competition or reproductive traits (e.g. flowering time or floral morphology) implicitly related to pollinator selection (e.g. Gould et al. 2013; Anderson et al. 2015; Berardi et al. 2016; Hirano et al. 2017, reviewed by Hargreaves et al. 2019). In contrast, studies of this nature addressing plant anti-herbivore defensive traits are rare, with only two recent exceptions involving reciprocal transplant studies showing that plant population differentiation in direct (Lowry et al. 2019) and indirect (Kergunteuil et al. 2019) defences along elevational gradients is associated with concomitant variation in plant fitness (see also Lowry et al. 2019). At the same time, whereas number of studies have measured plant-herbivore trait differentiation with respect to multispecies interactions under a spatial context (nonadditive or diffuse selection; Thompson 2005), most of this work has not involved ecological gradients, at least not explicitly. Finally, studies testing for latitudinal or elevational clines in phenotypic selection by herbivores on plants are virtually absent except for one study measuring selective effects of herbivory on plant reproductive output (Sandring et al. 2007), and we are aware of no studies measuring latitudinal or elevational variation in herbivore selection on putative plant defensive traits. Importantly, all of such studies have involved herbaceous plants, whereas tree species have been largely neglected.

Although common garden and reciprocal transplant experiments represent a powerful tool for testing plant adaptive differentiation to biotic and abiotic factors along geographical clines (Hargreaves et al. 2019), they are logistically demanding and impose constraints on the number of replicates that could ideally be established along the gradient, as well as replication within each location (Kawecki and Ebert 2004). In the case of long-lived plants, they may be further limited due to the difficulty of assessing lifetime or long-term measurements of fitness. Similarly, while it is also desirable to measure plant performance or fitness over several seasons to provide a more robust assessment of local adaptation, long-term measurements are often not feasible. More broadly, by testing for differences between selected points along the gradient, this approach also imposes a discretization of continuous ecological variation found along clines which may limit an assessment of the full range of trait variation and its underlying (continuous) drivers. On the other hand, regression-based methods used to estimate phenotypic selection on target traits are easier to implement methodologically and logistically, and a large number of estimates for multiple populations can allow to analyse continuous variation along a studied gradient. However, temporal replication of selection measurements is also desirable to better characterize selective regimes (Schemske and Horvitz 1989;

Siepielski et al. 2009; Ehrlén 2014), and even in the few cases where this has been addressed, inference about long-term patterns is often limited. Furthermore, intrinsic ecological noise in some systems may be substantial such that patterns of temporal variation in phenotypic selection may not be informative about observed adaptive population differentiation.

In addition to transplant experiments and regression-based measurements of phenotypic selection, the use of genetic markers has proven highly useful in relating genetic population differentiation with estimates of phenotypic selection on target traits or fitness measurements in experimental studies along ecological gradients (e.g. Lowry et al. 2019; Hirano et al. 2017; Schemske 1984; Anderson and Geber 2010). More recently, high-throughput sequencing has allowed to identify genomic regions, groups of genes, or specific genes underlying plants traits (reviewed by Bazakos et al. 2017), and these data can be analysed with respect to variation in biotic or abiotic factors to elucidate the genetic basis of adaptive differentiation along ecological clines (e.g. Tiffin and Ross-Ibarra 2014; Hoban et al. 2016; Wang et al. 2018). In particular, genomic tools can be used to measure the genetic signature of natural selection on targeted genes associated with plant resistance to herbivory across multiple populations (of a single species) or across species within a given clade. These methods, however, are currently unexploited in gradient studies (see De Mita et al. 2013; Dalongeville et al. 2018) and could represent a powerful option for assessing micro- and macro-evolutionary patterns associated with variation in biotic and abiotic selective pressures along latitudinal and elevational clines.

Elevational and Latitudinal Gradients in Plant Defences Through a Genomic Lens

We depart from the general observation that few studies have explored patterns of clinal selection on plant defensive traits at the molecular level (Züst et al. 2012; Brachi et al. 2015). Based on this, we elaborate on methodological and analytical approaches in evolutionary genomics (at micro- and macro- evolutionary levels) that can be used to unveil clinal patterns of adaptive evolution in plant defences (and, in the same way, plant enemy counterdefences). These methods can be applied to any plant species, though there are several criteria of taxa selection (see ahead) that can be followed to maximize the power of these analyses.

Assessing Micro-evolutionary Patterns of Clinal Variation in Plant Defences

Research on landscape genomics has played a fundamental role in detecting loci under selection and identifying environmental variables acting as selective sources while simultaneously considering genome-wide demographic effects such as gene flow, genetic drift, bottlenecks, and inbreeding (Schoville et al. 2012; Allendorf et al. 2010; Luikart et al. 2003; Stinchcombe and Hoekstra 2008). Broadly speaking, this approach involves in situ sampling of several individuals from multiple populations along a heterogeneous landscape (e.g. across contrasting habitats or along environmental clines), sometimes also involving ex situ sampling of source populations under controlled (e.g. greenhouse or common garden) conditions to minimize environmental effects (Fig. 18.2a, b) and the genotyping of all individuals at many independent loci (i.e. a genome scan). This scan may or may not include candidate genes underlying traits of interest (Fig. 18.2c). Following from this, the level of genetic

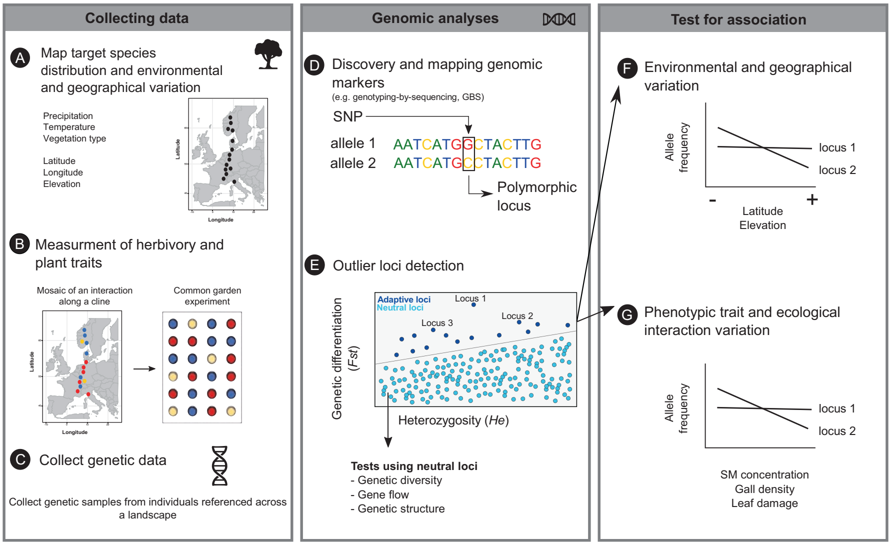

Fig. 18.2 Workflow depicting approach for testing latitudinal or elevational gradients in selection on target plant genes coding for putative defensive traits and their underlying correlates (i.e. herbivory and/or abiotic factors). (**a**) Collecting information about target species distribution along the gradient as well as geographical and environmental information. (**b**) In the field, sample individuals along the gradient to record phenotypic values for putative defensive traits (e.g. leaf toughness, trichome density, secondary metabolites concentration “SM”), measurements of antagonistic interaction strength (e.g. infection rate, amount of leaf damage). In parallel, collect seeds from sampled individuals in the field to conduct a common garden experiment to test for population differentiation in putative defensive traits as well as to conduct trait measurements to be used in testing for associations between allele frequencies, trait values, and environmental factors and herbivory (or pathogen infection). (**c**) Collect genetic samples. (**d**) Discover and map genomic markers using procedures such as genotyping-by-sequencing (GBS). (**e**) Use these data to perform an outlier loci test to detect candidate loci under selection. Non-outlier loci evolving under neutral evolution can be used in complementary tests. (**f**) A negative association between allele frequency of a locus previously detected under selection and latitude or elevation would be indicative of clinal variation in selection (i.e. as shown for locus 2). (**g**) An association between allele frequency for a given outlier locus and the phenotypic values (from field sampling or common garden experiment) of a putative defensive trait (e.g. SM) would provide evidence that a given locus codes for the plant trait measured (e.g. locus 2), whereas correlations between allele frequency and the amount of leaf damage or performance by herbivores or pathogens would suggest a role in resistance for the locus

differentiation across populations can be estimated (Fig. 18.2d; see Stinchcombe and Hoekstra 2008; Sork et al. 2013). With this approach, loci are assumed to evolve by natural selection if they are strongly differentiated among populations (outlier values of the coefficient of population differentiation, *F*st) when compared with selectively neutral regions (Fig. 18.2e; see Allendorf et al. 2010; Luikart et al. 2003). Next, statistical associations between allelic frequencies of loci detected under selection (i.e. loci with outlier *F*st values) and environmental variables can be used to unveil potential sources of selection (Fig. 18.2f, g). For more detailed technical considerations, see De Mita et al. (2013) and Dalongeville et al. (2018).

By applying the above procedure under a gradient-explicit framework, the frequency of alleles of outlier loci can be tested against herbivory (or plant pathogen infection) across populations as a preliminary assessment of their potential adaptive value in the context herbivore resistance, as well as with latitude or elevation to assess the magnitude and direction of clinal variation in allele frequencies (Fig. 18.2f, g). Likewise, multiple regression or structural equation modelling can also be used to assess correlations between allele frequencies and biotic factors (herbivory, pathogen infection), as well as abiotic (e.g. climatic) variables, and tease apart the relative influences of these environmental drivers (see 2.3 on the importance of considering abiotic factors). Further work with candidate loci significantly associated with latitude or elevation and with herbivory could include tests of their functional role in herbivore resistance by means of genome-wide associations with putative defensive traits, ideally under a common garden setting (e.g. de Villemereuil et al. 2016). In addition, gene mapping based on quantitative genetic approaches can be used to corroborate the genetic basis and function of such candidate genes by using linkage disequilibrium mapping and QTL mapping (Stinchcombe and Hoekstra 2008), as well as with gene knockout experiments (e.g. gene editing using CRISPR Bortesi and Fischer 2015).

This landscape genomics approach has been used for a number of tree species to discover candidate genes coding for traits associated with water-use efficiency, abiotic-related stress responses, as well as wood quality or density. Most of the traits looked at in these studies are linked to adaptation to abiotic factors (e.g. temperature, humidity) and to resource use and acquisition (De Kort et al. 2014; Eckert et al. 2010; Jaramillo-Correa et al. 2015; Manel et al. 2012). Similarly, a number of studies with herbaceous species have tested for associations between outlier loci and abiotic environmental variables in the context of adaptation to climatic change (Lee and Mitchell-Olds 2012; Bragg et al. 2015; Clark et al. 2007; Bergelson et al. 2001). Unfortunately, few of these studies have looked at genes and traits putatively associated with plant-enemy interactions (but see Brachi et al. 2015), despite the fact that these genes frequently exhibit high levels of polymorphisms across populations (Clark et al. 2007; Bergelson et al. 2001). Consequently, we know very little about the landscape genomics of plant-enemy interactions (Brachi et al. 2015), particularly under ecological gradients.

Employing landscape genomics research to uncover loci coding for plant traits presumed to be involved in herbivore (or pathogen) resistance in plants can provide a useful way forward to detect loci under selection, their role in herbivore resistance, and identify biotic (and/or abiotic) agents of selection, including different plant enemy taxa or guilds. Accordingly, as the number of elevational and latitudinal studies utilizing these approaches accrues, including work involving multispecies comparisons (e.g. Manel et al. 2012), we will start to gain knowledge on candidate genes, underlying traits, and ultimately a better understanding of clinal micro-evolutionary patterns in plant defence.

Assessing Macro-evolutionary Patterns of Clinal Variation in Plant Defences

The preceding section addressed genetic and genomic approaches at the population level to detect ongoing micro-evolutionary patterns of selection. We next introduce comparative approaches to detect signatures of selection at the species level that can inform on macro-evolutionary clinal patterns of adaptive evolution in plant defences.

Just as clinal variation in herbivore pressure has likely selected for concomitant clines in plant defensive gene variation within species (see Sect. 4.1), we could also expect a gradient in the signature of selection across species with contrasting latitudinal distributions (Fig. 18.3). Under this context, the expectation would be that rates of adaptive evolution in genes associated with plant defence are higher for species distributed at lower latitudes and elevations, as a consequence of more intense herbivore or pathogen selection on the defensive traits they code for. This prediction could be tested by comparing the rates of adaptive evolution of target genes underlying plant defensive traits across a number of plant taxa and from this infer clinal variation in the signature of selection on such genes. One way of measuring gene adaptive rates is in terms of the ratio of non-synonymous (dN, i.e. those affecting protein structure and function) to synonymous (dS, i.e. those not affecting protein function and evolve neutrally) mutations in such genes (Yang and Bielawski 2000; Li et al. 1985). If herbivore and pathogen pressure strengthen with decreasing latitude and elevation, then beneficial mutations in defensive genes should be more frequently fixed by selection in species found at lower latitudes and elevations. Therefore, non-synonymous mutations that increase the defensive value of a particular gene (i.e. substitution resulted in a beneficial mutation) should have a higher probability of spreading through a plant population and therefore become fixed more often at lower latitudes and elevations, than synonymous mutations (Yang and Bielawski 2000). Accordingly, the signature of selection estimated as the ratio $\omega = dN/dS$ should be higher than 1 at lower latitudes (e.g. for tropical vs. temperate lineages), i.e. selection favours fixation of non-synonymous mutations (dN) at a rate above neutral expectations (dS), a pattern indicative of so-called positive selection. Conversely, $\omega < 1$ would be indicative of “negative” or “purifying” selection which acts against (i.e. purging) non-synonymous mutations more strongly relative to neutral mutations. Finally, when $\omega = 1$ (i.e. equal rates of dS and dN), this indicates lack of selection on phenotypic variation at the codon level

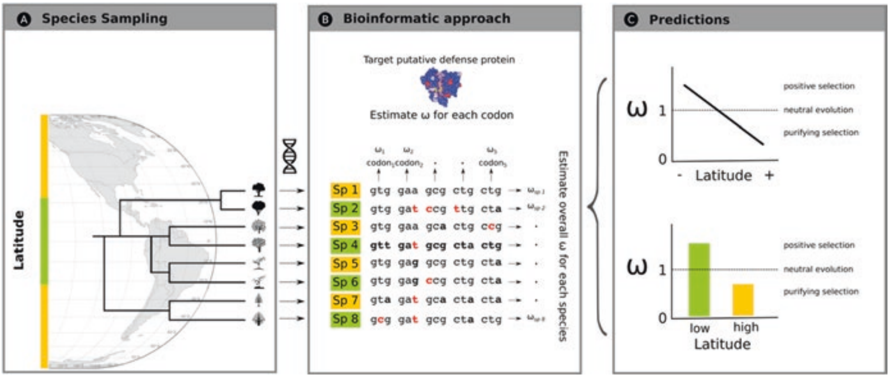

Fig. 18.3 (**a**) Sampling sister species with contrasting latitudinal or elevational distributions (minimizing species overlap as much as possible) while controlling for ecological and evolutionary factors (e.g. age, life form, effective population sizes, etc.). Species with tropical distributions are in green, whereas those with boreal and austral distributions are in yellow. (**b**) Sequencing and aligning orthologous target defensive gene (e.g. class I chitinase; protein illustration taken from Bishop et al. 2000; red dots within the protein denote detected sites under positive selection). Multiple alignment and phylogenetic tree are used by PAML to assess the signature of selection (ω = dN/dS) at the codon level (i.e. sites within protein assuming equal evolutionary rates across species; horizontal axis of variation) and species level (ω is averaged across codons within the sequence for each species). Nucleotide bases in bold indicate mutations, and non-synonymous mutations are denoted in red, whereas synonymous mutations are in black. (**c**) Upper panel shows a negative association between ω averaged estimates for each species (i.e. vertical axis of variation in B), and latitude or elevation will indicate that species distributed at higher latitudes or elevations have lower rates of adaptive evolution of defence genes than those found at lower latitudes or elevations. More sophisticated PAML models (e.g. branch-site and clade models) can incorporate both axes of variation to test for contrasting patterns of positive selection at the site level among different groups of species (e.g. grouped into low or high latitude based on their distribution). (**c**) Lower plot depicts results from a more detailed analysis combining site- and species-level variation (branch-site and clade models in PAML) which allows to explicitly test whether the signature of positive selection is higher for species evolving at lower latitudes than those evolving at higher latitudes

implying that non-synonymous mutations evolve neutrally (Yang and Bielawski 2000; Li et al. 1985).

Genomic and statistical tools have been used to test for latitudinal gradients in evolutionary rates of neutral genetic markers in plants (Wright et al. 2003, 2006; Davies et al. 2004), but to our knowledge have not been used to test for macroevolutionary patterns of clinal evolution for genes associated with anti-herbivore defences. To achieve this, we envision a procedure that would involve a phylogenetically controlled selection of plant taxa (e.g. congeneric species) with contrasting latitudinal or elevational ranges and with little overlap in their latitudinal or elevational distribution ranges (Fig. 18.3a; Wright et al. 2006; Gillman et al. 2009). The selection of candidate species should ideally also account when possible for other confounding factors such as life form (e.g. sample only tree species), mating system, and functional traits (e.g. shade tolerance, resource acquisition strategies).

Another important criterion would be to choose common species with large populations in order to control for effects of genetic drift caused by small effective population sizes (Wright et al. 2006). For each species, the same putative defensive ortholog gene (i.e. a gene that descends from a common ancestral form) must be sequenced to then perform a multiple alignment that is used, in combination with a phylogenetic tree of the studied taxa (Fig. 18.3a), to assess the signature of selection (ω) across species at the protein site level (i.e. codon) (Fig. 18.3b), as well as to compare the signature of selection among sites between previously determined groups of species (Yang and Bielawski 2000). Currently, the debate on methods to assess the molecular signature of selection is ongoing and leading to improvements of these statistical tools (Kosakovsky Pond and Frost 2005), and in some cases the best option has been to report results from simultaneously competing procedures (for a discussion on relevant methods, see Kosakovsky Pond and Frost 2005; Kosakovsky Pond et al. 2005). We next provide a closer look at one of these approaches based on Phylogenetic Analysis by Maximum Likelihood (PAML) (125), which is one of the oldest, most widely used, and best documented methods. It also has a relatively easy implementation (e.g. included in Biopython, and ETE tool) and is under constant update.

Specifically, PAML combines Markov chains and maximum likelihood to model the substitution process at the codon level and maximize the estimation of dN and dS and, consequently, ω (for details, see Yang and Bielawski 2000; Yang 2007). This approach can provide a detailed assessment of variation in ω among protein sites across species (Fig. 18.3b; free-ratio model (Nielsen and Yang 1998)), and also of variation in the signature of selection across sites within a protein to detect specific sites under positive selection and estimating the proportion of sites under positive selection (Fig. 18.3b). One way of testing whether defensive genes evolve faster at lower latitudes (or elevations) using this method would be to test for an association between the overall signature of selection estimated for each species ($\omega_{\mathrm{sp}\,1}\ldots\,\omega_{\mathrm{sp}\,n}$; using free-ratio models) against latitude or elevation (Fig. 18.3c, regression-type plot) (Yang 1998). It should be noted, however, that the signature of selection on protein sites under positive selection could be masked by strong purifying selection acting on most sites to maintain protein structure and function. This may lead to an underestimation of positive selection or to ambiguity in the interpretation of clinal patterns in the signature of selection, as values could result from weakening of purifying selection rather than due to positive selection (particularly when ω values do not exceed 1). Despite this caveat, evidence that ω increases (over and above 1) toward lower latitudes and elevations can be taken as good evidence of higher rates of evolution of putative defensive genes.

More advanced PAML models (branch-site and clade models) are capable of testing whether the pattern of ω at the site level within a protein is different between groups of species (Zhang et al. 2005; Weadick and Chang 2012). For example, previous studies have reported contrasting levels of positive selection on the visual pigment rhodopsin in cichlids species evolving in rivers vs. lakes (Schott et al. 2014) and on defensive protein class I chitinase between sexual and asexual *Oenothera* species (HerschGreen et al. 2012). We suggest that these types of models could be used to test whether specific plant defence genes differ in their rates of evolution in response to latitude or elevation by coding species as low- or high-latitude (or elevation) groups. The basic prediction would be that the overall estimation of ω for a defensive protein shared by a group of species from lower latitudes or elevations will be higher (w > 1, indicating positive selection) than the ω estimated for the same protein for a group of species distributed at higher latitudes or elevations (Fig. 18.3c, lower plot). In this case, and in contrast to the former approach based on regression-type analyses which requires limited overlap in latitudinal distributions among species (see above), one criterion for species selection could be that they naturally fall into different groups (low vs. high latitude) and within each group species would have roughly similar distributions in order to increase the power of this type of categorical analysis.

Codon-based models such as PAML as well as others such as HyPhy (Kosakovsky Pond et al. 2005) have been key to study the signature of selection on defensive genes implicated at different stages of the plant's immune system response (HerschGreen et al. 2012; Tiffin and Moeller 2006; Mondragón-Palomino et al. 2002; Brunner et al. 2013). Here we suggest that the same tools can be used to test for latitudinal and elevational hypotheses, for which there are clear predictions but contradictory empirical evidence. Genomic and bioinformatics tools can provide a large number of datasets to test at genome-wide scale (Carmona et al. 2019). The potential to reveal macro-evolutionary patterns of latitudinal gradient on defence genes at the genomic level (i.e. hundreds of defence genes) lies in the use of customized scripts that automatize PAML analyses or by using packages designed to run such analyses (Maldonado et al. 2016; Webb et al. 2017).

Integrating Species Interactions and Genomics Research in Gradient Studies

Our revision highlights considerable variation in patterns (i.e. direction) of elevational and latitudinal gradients in temperate tree defences and herbivory. Notably, only a third of the studies reviewed found support for the prediction of increased tree defences towards lower latitudes and elevations, though considerably more (about two thirds) found support for increasing herbivory towards lower latitudes. Likewise, only a third of the studies found higher herbivory at lower elevations. Moreover, patterns that are opposite to classic predictions, i.e. increasing defences and/or herbivory towards higher latitudes and elevations, were equally common and in some cases more common than expected patterns. These patterns are similar to results from previous meta-analyses including all plant life forms (e.g. Moles et al. 2011) and highlight that the current paradigm of geographical gradients in plant-herbivore interactions needs to be revised and calls for investigating likely sources of inconsistencies.

It is important to note that conclusions from qualitative reviews (such as this chapter) and meta-analyses should be made with caution given substantial differences across studies in experimental design and methodology, not to mention a number of other particularities of each study and model system used, including substantial differences in the latitudinal or elevational ranges of the studied species. Accordingly, recent work and our current analysis points to the necessity of increasing the methodological consistency across studies (e.g. herbivory and trait measurements (Anstett et al. 2016)), improving the methods for plant trait quantification (e.g. analytical resolution of chemical analyses (Anstett et al. 2016)), as well as a more comprehensive assessment of plant defensive phenotypes (e.g. multiple defensive traits (Moreira et al. 2018a)). In addition, we also argue that an explicit consideration of abiotic correlates of elevational and latitudinal gradients is essential.

As research on plant-herbivore latitudinal and elevational gradients closes these gaps by converging on similar methodologies as well as accounting for sources of biotic and abiotic variation, the adoption of genomic tools will open an unprecedented opportunity to launch a new generation of studies. One key realization is the need to merge research on landscape genetics with ecological studies of plant-intraspecific clines in plant-herbivore interactions. Surprisingly, landscape genetic work has barely been applied to plant defences (but rather mostly to traits mediating abiotic tolerance (Sork et al. 2013)) and has usually not explicitly addressed elevational and latitudinal gradients. A huge opportunity therefore lies for unlocking the potential of these genomic tools towards unveiling the genetic basis of clinal evolution in plant defences. Specifically, we envision future work on focal plant species coupling complementary approaches where consistent phenotypic measurements are taken along gradients and in common gardens with source populations, followed by genomic analyses to discover relevant and genetic manipulations to confirm the ecological and adaptive role of targeted plant defensive traits. In doing so, there is a need to fine-tune the criteria for taxa selection in order to increase the power of these studies while expanding this research to other tree taxa (e.g. under a phylogenetically controlled framework) that could be good models in order to reach reliable generalizations.

Species-level analyses of molecular evolution hold a similarly large potential for assessing plant defence macro-evolutionary patterns along environmental clines. Applying these proteomic-based evolutionary analyses provides a powerful approach to studying the genetic and molecular basis of plant defence macro-evolutionary patterns and in doing so test classic predictions about the evolutionary consequences of biotic and abiotic forcing along latitudinal or elevational clines. This includes tests of plant defence co-evolutionary models (e.g. Endara et al. 2017) and theory on rates of gene evolution (e.g. Wright et al. 2003, 2006) in the context of latitudinal and elevational gradients. In doing so, we can address whether rates of clinal evolution are faster for defensive vs. nondefensive (e.g. genes associated with physiological responses to abiotic stress or baseline rates (Wright et al. 2003)) and uncover sources of variation in the rates of clinal evolution of different families of defensive genes. Finally, results from applying these tools and methods to elucidate latitudinal or elevational macro-evolutionary patterns in plant defence can also contribute to develop new theory by uncovering patterns not apparent previously from studies based on measurements of plant traits and species interactions.

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