

Chemical communication and coevolution in an ant–plant mutualism

Martin Heil · Domancar Orona-Tamayo ·
Sascha Eilmus · Stefanie Kautz · Marcia González-Teuber

Received: 30 May 2009 / Accepted: 25 November 2009 / Published online: 15 December 2009
© Birkhäuser Verlag, Basel/Switzerland 2009

Abstract Protective ant–plant interactions provide valuable model systems to study mutualisms. Here, we summarise our recent research on chemical and physiological adaptations that contribute to the stabilisation of the mutualism between Mesoamerican *Acacia* host plants and their *Pseudomyrmex* ant inhabitants against exploiters, that is, species using host-derived rewards without rendering a service. *Acacia* hosts produce food bodies (FBs) and extrafloral nectar (EFN). Both types of reward are chemically adapted to their specific function as ant food and protected from different exploiters. FBs contained higher amounts of specific proteins than the leaves from which they originate. EFN possessed amino acids making it attractive for the mutualist ants and an invertase making its carbohydrate composition nutritionally suitable for the mutualists but unattractive for generalists. Moreover, pathogenesis-related proteins such as glucanases, chitinases and peroxidases were found in EFN, which likely serve as protection from microorganisms. Digestive adaptations were found that make workers of the ant mutualists dependent on the host-derived food sources, a mechanism that likely counteracts the evolution of cheaters. The ants also possessed a high diversity of bacterial associates,

several of which appeared involved in nitrogen fixation, thus contributing to the nutrition of these ‘vegetarian’ ants. By contrast, a non-defending ant species that parasitises the host plants appeared physiologically less adapted to the host-derived food rewards; this species, thus, likely is competitively inferior when colony growth is limited by plant-derived rewards. In summary, several physiological adaptations of both host plants and ants stabilise the *Acacia*–*Pseudomyrmex* mutualism against exploitation.

Keywords Cheater · Exploiter · Myrmecophyte · Indirect defence · Parasite of mutualism · PR-protein

Introduction

Mutualisms are interactions among partners of different species that benefit the fitness of all partners involved. In general, mutualisms are based on the exchange of resources: one partner typically provides a ‘service’ while the other partner, the host, provides a ‘reward’ (Bronstein 1994). Such rewards can also attract exploiter species, which use the rewards without returning a benefit, hence acting as ‘cheater’ or ‘parasite’ of the mutualism (Bronstein 2001; Douglas 2008; Sachs and Simms 2006). Exploiters have been described for several horizontally transmitted mutualisms, that is, mutualisms in which the symbiosis has to be established anew in every generation; examples include defensive ant–plant mutualisms (Clement et al. 2008; Gaume and McKey 1999; Izzo and Vasconcelos 2002; Janzen 1975; Raine et al. 2004), the nodulation of plant roots by nitrogen-fixing rhizobia (Denison and Kiers 2004; Kiers et al. 2003), and mycorrhizal associations (Hoeksema and Kummel 2003) (see Bronstein 2001; Sachs and Simms 2006; Yu 2001 for reviews).

M. Heil (✉) · D. Orona-Tamayo
Departamento de Ingeniería Genética, CINVESTAV-Irapuato,
Km. 9.6 Libramiento Norte, CP 36821 Irapuato, Guanajuato,
Mexico
e-mail: mheil@ira.cinvestav.mx

S. Eilmus · S. Kautz · M. González-Teuber
Department of General Botany-Plant Ecology, FB BioGeo,
University of Duisburg-Essen, Universitätsstraße 5,
45117 Essen, Germany

Exploiters can decrease the net benefit that hosts obtain from producing rewards and do not pay the cost of providing services. Many authors have, thus, suggested that exploiters should be competitively superior to mutualists (Doebeli et al. 2004; Doebeli and Knowlton 1998; Kiers et al. 2003; Sachs and Simms 2006). As a result, many theories revealed the prediction that exploiters will invade mutualisms and impose selection on hosts for reduced reward levels, finally causing the evolutionary dissolution of the mutualism (Bronstein 2001; Doebeli and Hauert 2005; Ferrière et al. 2007; Hoeksema and Bruna 2000; Sachs et al. 2004). However, in an obvious contradiction to theoretical expectations, few phylogenetic studies have found evidence for the existence of exploiters with mutualistic ancestors (Douglas 2008; Sachs and Simms 2006). This observation led to the prediction of mechanisms that can stabilize mutualisms.

Because the phylogenetic history of an exploiter determines the mechanism that can stabilize a mutualism against the evolution and maintenance of the exploiter (Bronstein 2001), we hereinafter use the term ‘cheater’ explicitly for exploiters that have evolved from former mutualists (Segraves et al. 2005), while species that invaded the system without having an evolutionary history as a mutualist are termed ‘parasites’ (Bronstein 2001; Heil et al. 2009; Kautz et al. 2009a). The evolutionary persistence of horizontally transmitted mutualisms is generally attributed to host sanction mechanisms (Bergstrom and Lachmann 2003; Bull and Rice 1991; Foster and Wenseleers 2006; Sachs et al. 2004), that is, host behaviours that allow a preferential provisioning of rewards to reciprocating, mutualistic partners, while exploiters receive less or no rewards. Striking examples are represented by yucca plants, which abort flowers that contain too many eggs of the pollinating (but also florivorous) moths (Pellmyr and Huth 1994), and legumes, which reduce the allocation of assimilates to nodules that contain non-nitrogen-fixing bacteria (Kiers et al. 2003).

Mutualisms can also be stable when partner choice mechanisms allow the preferential association of hosts with mutualists, that is, when hosts can judge the quality of potential partners before the interaction is established (Bull and Rice 1991; Foster and Wenseleers 2006). Indeed, some ant–plants restrict the provisioning of housing or food rewards to co-evolved (and mutualistic) ant species (Brouat et al. 2001; Federle et al. 2001; Heil et al. 1997; Risch and Rickson 1981), and client reef fishes avoid visiting cleaner fishes that feed on client mucus rather than the client’s ectoparasites (Bshary and Grutter 2005). The main difference between host sanctions and partner choice lies in the mechanisms by which the host identifies the quality of the partner and in the phase of the establishment of the mutualism at which the mechanism becomes active: host

sanctions occur once a symbiosis has been established and require that hosts are able to monitor the performance of their symbionts, whereas partner choice mechanisms are based on other characteristic traits of the potential symbionts and select these before a symbiotic interaction is established. Unfortunately, our understanding of the underlying chemical and ecological mechanisms is generally scarce, with the sole exception of plant–rhizobia interactions, where both mechanisms have been described: detailed chemical signalling cascades allow only selected bacterial strains to successfully infect plant roots (Limpen and Bisseling 2003; Mithöfer 2002) and host sanction mechanisms monitor the performance of bacteria once nodules have been established (Kiers et al. 2003; Simms et al. 2006; West et al. 2002).

The aim of the present review is to summarise the recent developments in our research on one particularly striking example of mechanisms that prevent the evolution of cheaters and exclude parasites from exploiting a mutualism: reciprocal adaptations of Mesoamerican *Acacia* host plants and their mutualistic *Pseudomyrmex* ant inhabitants, a defensive ant–plant mutualism. For ant–plant mutualisms, two biologically different types of exploiters have been described. Some ant species, which have been termed ‘castration parasites’, remove the flowers of their host plants in order to divert more resources to vegetative growth and, thus, ant reward production (Gaume et al. 2005; Izzo and Vasconcelos 2002; Yu and Pierce 1998). Other exploiting ants (including the case that forms the focus of the present study) only make use of the host-derived resources without providing the protection service, but they do not actively damage the host (Clement et al. 2008; Janzen 1975; Raine et al. 2004; Tillberg 2004). According to the phylogenetic definitions suggested above, all these ant species would represent parasites, as no evidence has been presented that one of them has evolved from a mutualistic ancestor. The *Acacia*–*Pseudomyrmex*-system appeared particularly suitable to study mechanisms that stabilize the mutualism against parasitisation, because preliminary field observations revealed that different host plant species suffer from parasitisation at very different rates: natural variation among species did, thus, allow a comparative approach to search for purportedly stabilizing traits, or mechanisms, on the side of the host plants.

Study system

Our study system consisted of four Central American myrmecophytic *Acacia* species that live in an obligate protection mutualism with ant species of the *Pseudomyrmex ferrugineus* group (Janzen 1966). Central American ant–acacias provide their ants with nesting space in hollow

thorns and with food rewards: extrafloral nectar (EFN) and cellular food bodies (FBs) (Fig. 1). The plant species considered were *A. hindsii* Benth., *A. cornigera* (L.) Willdenow, *A. chiapensis* Safford and *A. collinsii* Safford. Species were determined by comparison with specimens at the Herbario MEXU at UNAM (Universidad Autónoma de México, Mexico City) and following Janzen (1974).

The mutualistic ant species *P. ferrugineus* F. Smith, *P. mixtecus* Ward and *P. peperii* Forel protect their hosts from herbivores and encroaching or competing vegetation (Fig. 1) and cannot be found nesting apart from their host plants (Clement et al. 2008; Janzen 1966). Two other ant species (*P. gracilis* Fabricius and *P. nigropilosus* Emery)

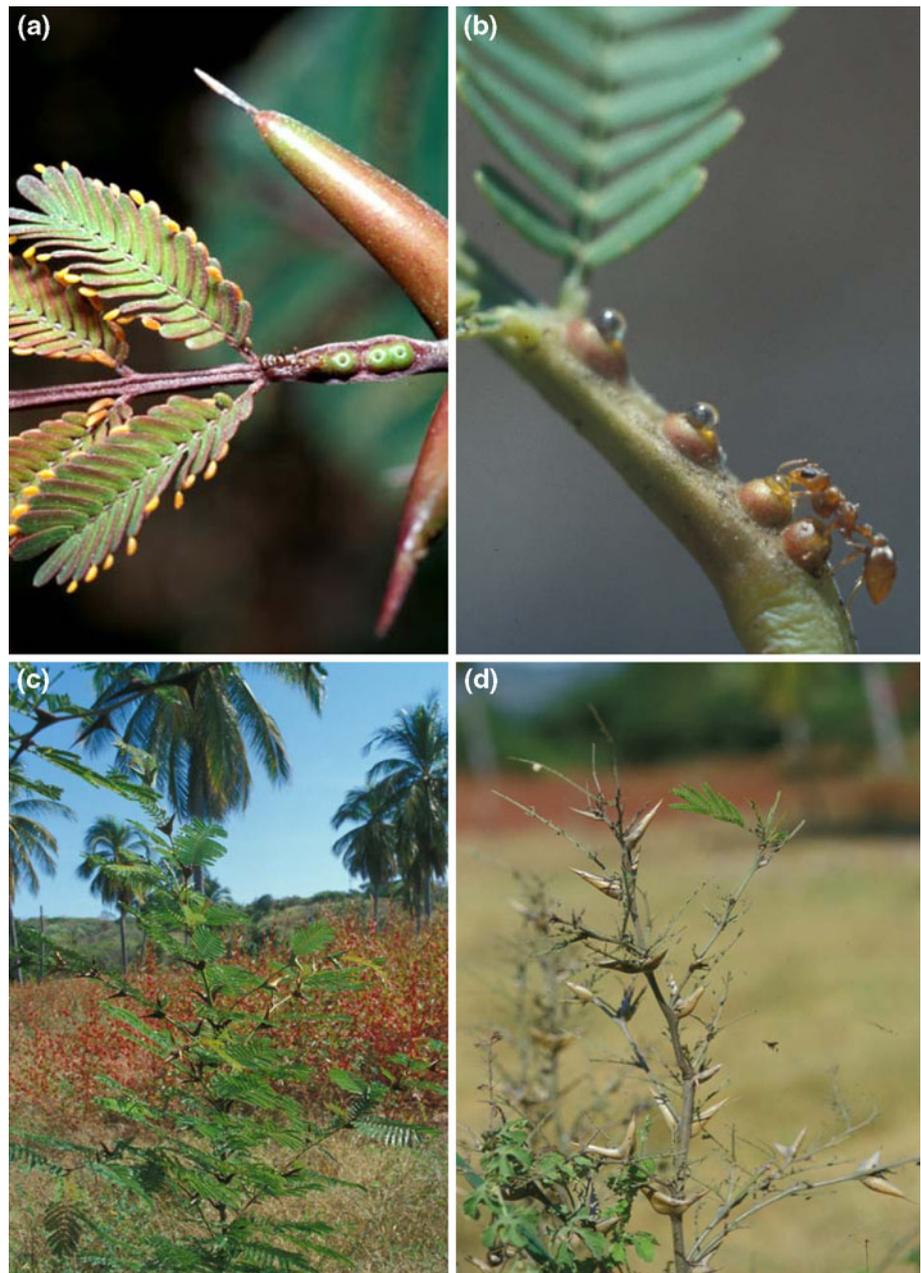
inhabit the hollow thorns and consume FBs and EFN but show no detectable defending behaviour, hence acting as exploiters of the mutualism (behavioural details for *P. nigropilosus* have been reported by Janzen (1975)). Ant species were determined according to Ward (1993).

Chemical adaptations on the plant side

Extrafloral nectar

Extrafloral nectar has now been described for species that belong to more than 300 genera of plants (Bentley 1977;

Fig. 1 The mutualism of Mesoamerican *Acacia* myrmecophytes with *Pseudomyrmex* ants. **a** Mesoamerican *Acacia* myrmecophytes such as *A. collinsii* provide their resident ant mutualists with cellular food bodies (yellow grains on leaflet tips) and extrafloral nectar. **b** *P. peperii* ant feeding on *A. hindsii* extrafloral nectaries. **c** *A. hindsii* plant inhabited by mutualist ants. **d** Ant-free *A. hindsii*. **a** Reproduced from Heil (2008)



Koptur 1992; see also URL: www.biosci.unl.edu/emeriti/keeler/extrafloral/worldlistfamilies.htm). Most plant species secrete EFN to attract defending ants as a means of indirect defence and are therefore commonly called 'myrmecophilic' (that is, 'ant-loving', for reviews see Heil 2008; Heil and McKey 2003). In general, EFN secretion represents an induced response to herbivory (Heil et al. 2004b, 2001b; Kobayashi et al. 2008; Mondor and Addicott 2003; Pulice and Packer 2008; Rogers et al. 2003; Wäckers and Wunderlin 1999; Wooley et al. 2007). By contrast, the obligate myrmecophytes among the Mesoamerican *Acacia* species secrete EFN constitutively at high rates to nourish their symbiotic ant inhabitants (Heil et al. 2004b). Myrmecophyte EFN appears, thus, in danger of being exploited by non-defending species and can be expected to be specifically adapted to the nutritive needs of the mutualistic *Pseudomyrmex* species.

Nectar compounds that are regarded as mainly responsible for the attraction of ants are mono- and disaccharides and amino acids (Baker and Baker 1975; Baker et al. 1978; Blüthgen and Fiedler 2004; Lanza et al. 1993). Analyses of these quantitatively predominant component classes in *Acacia* EFN revealed that myrmecophyte EFN contained only the two monosaccharides, glucose and fructose, whereas EFN of myrmecophilic species contained these two monosaccharides and also the disaccharide, sucrose (González-Teuber and Heil 2009; Heil et al. 2005). Sucrose has proven attractive to many generalist ants (Blüthgen and Fiedler 2004; Boevé and Wäckers 2003; Cornelius et al. 1996; Stapel et al. 1997; Vander Meer et al. 1995), but mutualistic *Pseudomyrmex* species preferred sucrose-free EFN in various, independently conducted cafeteria-style experiments (Fig. 2; González-Teuber and Heil 2009; Heil et al. 2005). Moreover, the amino acid fraction of EFN of the myrmecophytes, *A. cornigera* and *A. hindsii*, was quantitatively dominated by leucine, phenylalanine, proline and valine, and mutualist ant workers preferred EFN mimics containing exactly these four amino acids over mimics that contained more or fewer amino acids (Fig. 2), while generalist ants preferred EFN mimics with a higher number of different amino acids (González-Teuber and Heil 2009). Attracting non-defending or non-specialised EFN visitors can reduce the defensive efficacy of EFN secretion (Heil et al. 2004c; Mody and Linsenmair 2004), whereas the attraction of adult EFN-feeders with herbivorous larval stages (Beach et al. 1985) might even cause significant ecological costs. The same 'conflict' applies to other mutualisms, for example, when pollinators possess herbivorous larvae that feed on the leaves of the same plant species that is, or are, pollinated by the adult animal (Bronstein et al. 2009).

Thus, we conclude that the sugar and amino acid composition of myrmecophyte EFN is chemically tailored to the preferences of the specialised mutualist ants and at the

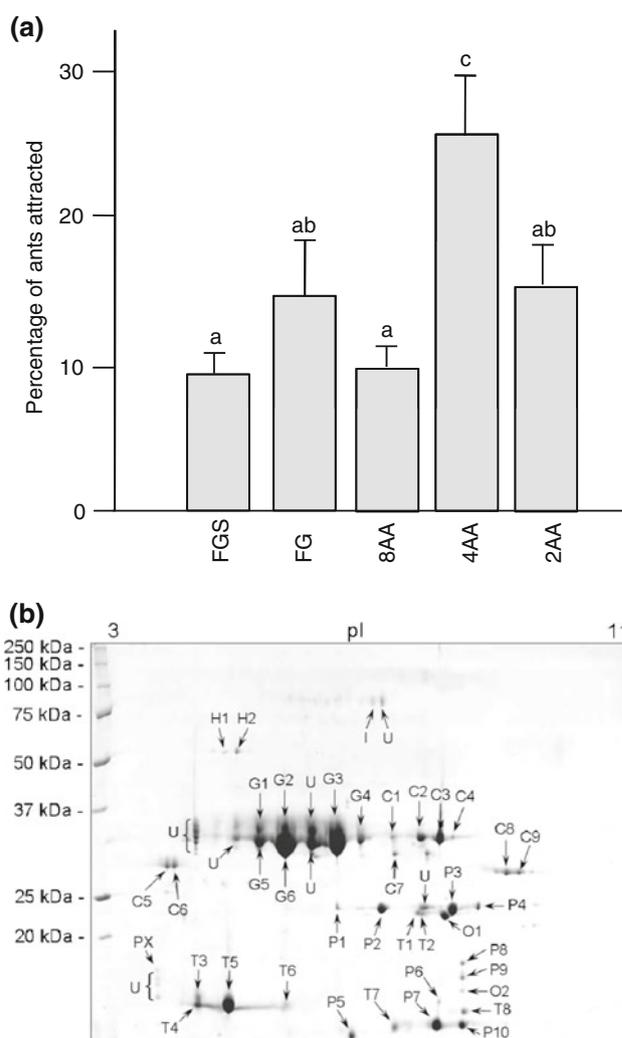


Fig. 2 Chemical adaptations of myrmecophyte EFN. **a** Percentage of mutualist workers attracted in cafeteria-style experiments to different nectar mimics (FGS fructose, glucose and sucrose, FG fructose and glucose, 8AA FG + eight amino acids, 4AA FG + four amino acids, 2AA FG + 2 amino acids; see (González-Teuber and Heil 2009) for details). **b** The proteome of *A. cornigera* EFN (C chitinase proteins, G glucanase proteins, H glycoside hydrolase proteins, I invertase protein, O osmotin proteins, P PR-proteins, PX peroxidase proteins, T thaumathin-like proteins, U unknown proteins). Figure redrawn from data published in González-Teuber and Heil (2009) and González-Teuber et al. (2009)

same time makes this EFN less attractive to generalists that might function as potential exploiters. This interpretation was supported by the finding of an invertase activity in the myrmecophyte EFN, which hydrolyses sucrose (Heil et al. 2005). Although present only in low amounts (Fig. 2), this invertase was found to be highly active and sufficient to obtain a complete post-secretory hydrolysis of sucrose in the EFN of myrmecophyte *Acacia* species (Heil et al. 2005).

Although the specific sugar and amino acid composition of the EFN of myrmecophytic *Acacia* species contributes

Table 1 Relative protein contents of extrafloral nectar

	Myrmecophytes		Myrmecophilic species	
	<i>A. cornigera</i>	<i>A. hindsii</i>	<i>A. farnesiana</i>	<i>Prosopis</i>
Proteins [$\mu\text{g mg}^{-1}$ sugars]	185 \pm 19	222 \pm 49	13 \pm 4	11 \pm 15

Table 2 Protein contents of leaves and FBs

	Food bodies		Leaves	
	<i>A. cornigera</i>	<i>A. hindsii</i>	<i>A. cornigera</i>	<i>A. hindsii</i>
Proteins [$\mu\text{g mg}^{-1}$ dw]	241 \pm 8	213 \pm 26	86 \pm 4	84 \pm 3

to its effective protection from insect exploiters, EFN still appears a highly suitable medium for microbial growth. Yeasts in particular have been reported from nectars (Brysch-Herzberg 2005; Herrera et al. 2009; Sandhu and Waraich 1985) and might significantly affect their carbohydrate composition (Herrera et al. 2008), hence reducing the chemical control of the plant over this important trait. When we studied microbes present in field-derived samples of EFN, we were, however, unable to cultivate any yeasts or fungi from nectar of the two myrmecophytes, *A. cornigera* and *A. hindsii*, although these microorganisms could be successfully isolated from EFN of myrmecophilic plants that were growing at the same site (González-Teuber et al. 2009). Bioassays demonstrated that yeast growth is significantly inhibited in myrmecophyte EFN as compared to mere sugar solutions and EFN of the myrmecophilic species. A more detailed investigation of the protein fraction of myrmecophyte EFN revealed the presence of glucanases, chitinases, peroxidases, thaumatin-like proteins and other pathogenesis-related (PR-) proteins (González-Teuber et al. 2009), which repeatedly have been demonstrated to serve as plant defences against fungi and other microorganisms (for reviews see Van Loon et al. 2006; Van Loon and Van Strien 1999). Interestingly, myrmecophyte EFN contained much more proteins than EFN of myrmecophilic species (Table 1). Thus, the protein fraction of EFN functions in the protection of EFN from microbial infestation, as has already been reported for floral nectar (Carter and Thornburg 2004; Nicolson and Thornburg 2007; Thornburg 2007; Thornburg et al. 2003).

Food bodies

Besides EFN, Mesoamerican *Acacia* myrmecophytes provide their ant inhabitants with a second type of reward, FBs (Fig. 1), which are produced during leaf development on the leaflet tips. Although FBs are thus ontogenetically derived from leaf tissue (Rickson 1969, 1975) we found contents of nutritionally valuable proteins and lipids in

their tissue that by far exceeded the contents of the leaf tissues (Heil et al. 2004a) (Table 2). A more detailed analysis of the FB proteome revealed that the protein fraction of *A. hindsii* FBs differed not only quantitatively but also qualitatively from the leaves of the same species (unpublished results by D. Orona-Tamayo): the majority of proteins that could be found in the FBs were different from the leaf proteins (Fig. 3). Although detailed analyses are still underway, this pattern indicates that the FB proteome of Mesoamerican *Acacia* myrmecophytes is adapted to serve the FBs function as the main (or only) food source of the larvae of the specialised ant inhabitants.

Physiological adaptations of the ant workers

Digestive capacities: invertase

As reported above, the EFN of *Acacia* myrmecophytes contains an invertase and thus is kept free of sucrose. Although sucrose is attractive to many ant species, the specialised *Pseudomyrmex* workers preferred this EFN over sucrose-containing solutions (Heil et al. 2005). Why do these ants reject a disaccharide that is highly attractive to most ant species? Studying invertase activity in the digestive tracts of these ants revealed that workers of *Pseudomyrmex* ant exhibited only extremely low activity of invertase and were even unable to increase the activity of this digestive enzyme when sucrose was present in their diet (Fig. 4). By contrast, workers of exploiting species and of generalists possessed a substrate-inducible invertase (Fig. 4), as did the larvae of all investigated species (Kautz et al. 2009a). A phylogenetic analysis revealed that invertase-free workers occur exclusively in one clade within the genus *Pseudomyrmex*, which comprises the specialised species of the *P. ferrugineus*-group (Kautz et al. 2009a): these ants lost the capacity to digest the most common water-soluble carbohydrate in plants, sucrose, in the worker stage and thus appear physiologically

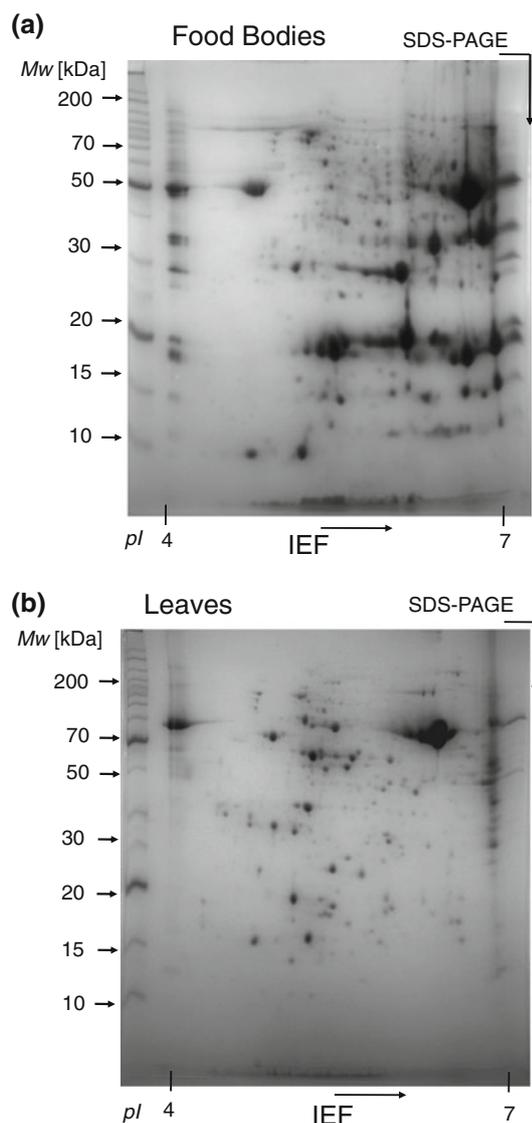


Fig. 3 The proteome of *A. hindsii* food bodies and leaves. Comparing the protein patterns as revealed by two-dimensional gel electrophoresis reveals that some 80% of the proteins that dominate the food body proteome (a) are different from leaf proteins of the same species (b). The first dimension was carried out by running 150 μ g of protein into 13-cm IPG strips, pH 4–7, and the second dimension was 13% of SDS–PAGE. Original gel size was 16 \times 18 \times 0.15 cm. The proteins were detected by Coomassie blue stain (D. Orón-Tamayo, unpublished results)

dependent on the ‘pre-digested’ EFN that is produced by their host plants (Heil et al. 2005).

Digestive capacities: protease

Analyses of stable isotopes had revealed that the natural abundances of ^{13}C and ^{15}N in specialised *Acacia*–ants were very close to those of the host plants from which ant workers and larvae had been collected, whereas the

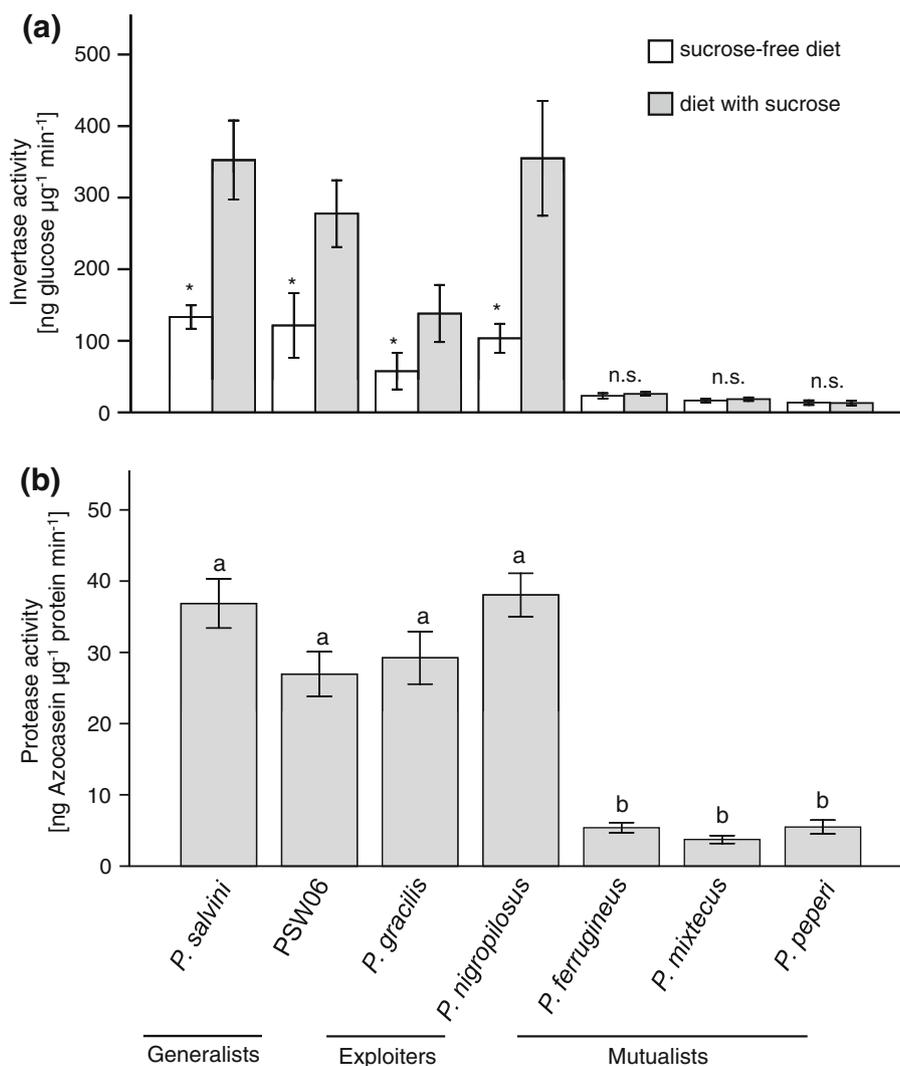
exploiter, *P. gracilis*, exhibited significantly enriched ^{13}C and ^{15}N signatures. This finding, together with behavioural observations, led to the conclusion that mutualistic *Pseudomyrmex* species obtain their entire food from their host plants, whereas the exploiting species also make use of host-independent food sources such as captured insects (Clement et al. 2008). We, thus, speculated that the capacity of protein digestion in mutualist workers might also differ from that in less specialised species. General protease activity was quantified in ant workers by preparing a crude extract of the workers’ digestive tracts and checking its proteolytic activity using a sulfanilamide–azocasein solution as the substrate (S. Kautz, unpublished data). Indeed, the ‘vegetarian’ mutualist workers exhibited significantly lower protease activities than workers of generalist and exploiting species (Fig. 4b).

Although two of the ant species that have been described as exploiters of Mesoamerican *Acacia* species belong to the same genus as the mutualists, a phylogenetic analysis revealed that both *P. gracilis* and *P. nigropilosus* have evolved their association with *Acacia* myrmecophytes without having a mutualistic ancestor (Kautz et al. 2009a). Because no cheaters have, thus, been described for this system, we conclude that the digestive specialisations described here make mutualist ants highly dependent on their specific host plants and, thus, represent a mechanism that prevents the evolution of cheaters. Due to the necessity to feed exclusively on host-derived food sources, an ant mutualist that starts to reduce its protective services would suffer from fitness disadvantages, because its performance is dependent on the state of its host (Heil et al. 2009).

Bacterial associates of the ants

Obligate plant–ants that feed only on host-derived rewards represent a particularly extreme case of nutritional specialisation. However, ‘vegetarian’ ants, which feed exclusively on food sources that are derived from plants, might be much more common than is generally assumed. Ants are the dominant insect group and can comprise more than 80% of the arthropods in tropical rain forests (Davidson and Patrell-Kim 1996; Hölldobler and Wilson 1990; Tobin 1995). Because ants thus reach much higher biomass values than their supposed prey organisms, it appears difficult to understand how ants can reach this high abundance, a phenomenon that has been termed ‘Tobin’s ant-biomass paradox’ (Davidson and Patrell-Kim 1996; Tobin 1995). Davidson et al. (2003) suggested that dominant ant taxa might be ‘more herbivorous’ than originally assumed and reported evidence from studies on $\delta^{15}\text{N}$ -ratios of ants from Amazonia and Borneo, which demonstrated that many arboreal ant species obtain little nitrogen through predation. Because plant-derived food sources appear too

Fig. 4 Physiological adaptations of *Acacia*-inhabiting ant mutualists. **a** Invertase activity in ant workers was expressed as nanogram glucose released from sucrose per microgram ant protein and minute separately for ants fed on a sucrose-containing or a sucrose-free diet over 5 days. Asterisks indicate significant differences between ants feed with and without sucrose ($P < 0.05$ with $N = 10$, except PSW06 where $N = 4$; Wilcoxon signed-rank test). **b** Protease activity was quantified as nanogram azocasein degraded per microgram ant protein and minute. Bars marked by different letters different at $P < 0.05$ (LSD posthoc-test after univariate ANOVA). Bars represent means \pm SE. Both types of enzymes were quantified in the digestive tract of the generalist species, *P. salvini* and the yet undescribed species *P. spec.* PSW-06, of the parasites *P. gracilis* and *P. nigropilosus* and of the mutualist species, *P. ferrugineus*, *P. mixtecus* and *P. peperi*. **a** Reproduced from (Kautz et al. 2009a), **b** drawn from unpublished data by S. Kautz



low in nitrogen as to allow the construction of the ants' bodies, the question remained open, however, how these 'vegetarian' ants can obtain a balanced alimentation.

Many insects depend on symbiotic bacteria for their nutrition (Chandler et al. 2008; Nardi et al. 2002; Zientz et al. 2006) and one possible explanation of Tobin's anti-biomass paradox would, thus, be the existence of anti-microbe mutualisms. If, for example, the ants have access to N that is fixed by bacteria directly from the atmosphere one would expect the $\delta^{15}\text{N}$ ratio of the ants to drop below the level that would be expected from a predatory and perhaps even a fully herbivorous organism. Because the mutualist, *P. ferrugineus*, lives on a strictly host plant-derived diet and possessed $\delta^{15}\text{N}$ ratios that were closer to those of its host plant than the 3.4‰ that would represent a full trophic level (Clement et al. 2008), this species appeared particularly suitable to search for the putative presence of N-fixing or N-recycling bacteria in an ant. Unfortunately, the discovery of yet unknown bacteria is severely hampered by

methodological restrictions: we lack suitable cultivation protocols for many environmental and endosymbiotic bacteria. Bacteria such as *Blochmannia*, *Buchnera*, *Rickettsia* and *Wolbachia* are extremely common intracellular endosymbionts of insects, at least when considering specific taxa (Dean 2006; Hilgenboecker et al. 2008; Perlman et al. 2006; Stoll et al. 2007; Werren et al. 1995), but many further microbial symbionts of insects likely remain to be discovered. Therefore, we applied an in silico analysis of terminal restriction fragments (tRF) for the gross characterisation of the microbial community of *P. ferrugineus* (Eilmus and Heil 2009). In short, we used fluorescence-labelled primers to amplify the bacterial 16S rRNA genes in samples obtained from extracted ants and the amplification products were then digested with restriction enzymes. The resulting tRF patterns usually are characteristic at the level of genera (Eilmus and Heil 2009; Roesch and Bothe 2005).

We found tRF patterns representing at least 30 prokaryotic taxa. Parallel analyses of environmental samples

and negative controls made sure that the majority of bacterial taxa found in our samples had indeed been obtained from the ants themselves, rather than representing environmental contaminations. Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Planctomycetes, Proteobacteria and Spirochaetes comprised more than 90% of the overall bacterial diversity detected. Genera for which a function as ant symbionts appeared most likely were *Burkholderia*, *Pantoea*, *Weissella* and several Enterobacteriaceae. Most interestingly in the present context, we detected seven taxa of potentially N-fixing organisms (Rhizobiales, Burkholderiales, Pseudomonadales, Spirochaeta, Cyanobacteria and the two genera of γ -Proteobacteria: *Pantoea* and *Serratia*), several of which could be confirmed by independent cultivation and sequencing techniques (Eilmus and Heil 2009). Moreover, tRF patterns consistent with the presence of *Buchnera*, an N-recycling bacterium, were present in all ant samples analysed. Although final evidence for the role of these bacteria is still lacking, these results provide further support for the hypothesis that ‘vegetarian’ arboreal ants might supplement their diet with nitrogen that is obtained from associated N-fixing and N-recycling bacteria.

Regulation of the production of ant rewards

As mentioned in the “Introduction”, mutualisms can be stabilised by partner choice or host sanction mechanisms, which enable the host to select suitable symbionts or to preferentially provide rewards to partners that render the expected service. For ant–plant interactions, a partner choice mechanism has been described for the genus *Leonardoxa* (Brouat et al. 2001). Obligate myrmecophytes in this genus provide nesting space to mutualist ants in domatia that open by prostomata, that is, pre-formed entrance holes. In each species, the prostomata had different shapes, and a significant correspondence among the morphology of the prostomata and morphological traits of the associated ant mutualists could be demonstrated (Brouat et al. 2001). Similarly, several ant–plants produce food rewards only when ants are present (Heil et al. 1997; Risch and Rickson 1981). Because reward production in some cases could also be induced by other, non-defending insects or by artificial removal of the rewards (Folgarait et al. 1994; Heil et al. 2000; Letourneau 1990), a chemical or mechanical signal, rather than partner performance, most likely triggers reward production by the host. Unfortunately, what signals are exchanged between host plant and reward consumer in order to elicit this response has remained an open question.

More recent evidence demonstrated, however, that some host plants indeed are able to reduce domatia production

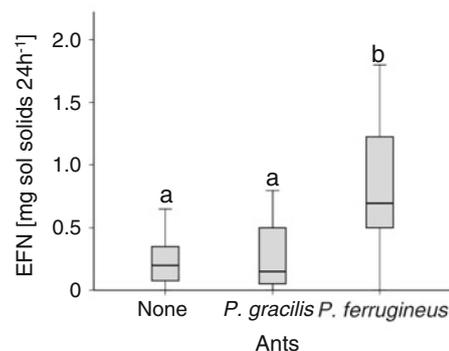


Fig. 5 Effect of ant species on EFN secretion. The EFN secretion on main shoots of *A. hindsii* plants was quantified as milligram soluble solids per 1 day separately for ant-free plants ($n = 15$) and plants inhabited by the mutualist, *A. ferrugineus* ($n = 20$) and by the parasite, *P. gracilis* ($n = 10$). Bars marked by different letters are different at $P < 0.05$ (LSD posthoc-test after univariate ANOVA). Figure redrawn from data presented in Heil et al. (2009)

when inhabited by non-defending or castrating ants and can therefore sanction against these exploiters (Edwards et al. 2006; Izzo and Vasconcelos 2002). Similarly, the rates at which *Acacia* myrmecophytes provide their resident ants with EFN depended on the ant species that was present on the plant (Heil et al. 2009). We selected *A. hindsii* plants inhabited by *P. ferrugineus* ($n = 20$), by *P. gracilis* ($n = 15$) or that were ant free ($n = 10$) and quantified EFN secretion on the main shoot as described previously (Heil et al. 2000, 2004b) as milligram of soluble solids secreted per 24 h. The presence of the mutualist, *P. ferrugineus*, had a significant positive effect on EFN secretion, while EFN secretion on plants inhabited by the parasite did not differ from that by controls (Fig. 5). Thus, the plants apparently sanction against the parasite. Because the parasite does not defend its host plant whereas the mutualist does, and because the experiment was conducted under natural herbivore pressure, this design did not allow separating mechanisms based on a chemical identification of the ant species from ‘true’ host sanction mechanisms, which in this case would act via a monitoring of the ants’ protective efficacy by the host plant and an according regulation of EFN secretion. However, these data demonstrate that parasitic ants receive fewer resources from their *Acacia* host than do mutualists, a phenomenon that likely stabilizes the association of hosts with mutualists against invasion by parasites or the evolution of cheaters. Moreover, a competition among mutualists and parasites appears to stabilize the co-existence of both types of ants (Heil et al. 2009), as it has earlier been suggested for other mutualisms (Morris et al. 2003).

Studies on various ant–plant mutualisms have demonstrated that ant protection increases the sizes of the host plants and, thus, food reward levels (Heil et al. 2004b), that

increased host plant size and food rewards increase ant-colony size (Edwards et al. 2006; Fonseca 1993; Heil et al. 2001a) and that the development of larger ant colonies improves the protection from herbivores (Duarte Rocha and Godoy Bergallo 1992; Heil et al. 2001a; Ness et al. 2006) and competitors (Palmer 2004). All these relations lead to host sanction phenomena, although they are likely results of the dependency of obligate plant–ants on the food and nesting space that is derived from their host, rather than being controlled by specific mechanisms.

Adaptations in ant-colony structure

Obligate ant–plant mutualisms suffer from the intrinsic problem that the potential lifetime of the host plant is usually longer than that of the inhabiting ant colony. At least some ant species have, however, ‘solved’ this problem via the evolution of polygyny, that is, an individual colony possesses more than one queen (Dalecky et al. 2005; Feldhaar et al. 2000; Janzen 1975; Kautz et al. 2009b). In fact, colonies of the obligate acacia–ant *Pseudomyrmex veneficus* may comprise hundreds of thousands of queens and millions of workers (Janzen 1975), and are among the largest of all social insect societies (Ward 1993). Therewith, the lifetime of a colony exceeds the lifetime of a single ant queen.

However, kin selection (Hamilton 1964) should favour ant colonies that possess one singly mated queen and are, thus, characterised by high relatedness among nestmates. When we employed microsatellite markers (Kautz et al. 2009c) to study the genetic colony structure of the highly polygynous acacia–ant, *P. peperi*, we found at maximum three alleles per locus within a colony although we had included queens, workers, males and virgin queens. The high relatedness among all colony members indicates that colonies had likely been founded by one singly mated queen and that polygynous colonies develop as a consequence of intranidal mating among males and daughter queens, which represent direct offspring of the same original queen. This strategy allows colonies to grow by budding and to occupy individual plant clusters for time spans that are longer than an individual queen’s life, while at the same time maintaining high nestmate relatedness (Kautz et al. 2009b).

Discussion and conclusions

Phenotypic plasticity is the ability of an organism to express different phenotypes in response to changing environments (Sultan 2000). Many mutualisms are based on long-term associations of the same individual partners.

The resulting possibility of iterated interactions among phenotypically plastic partners can stabilise mutualisms (Agrawal 2001; Doebeli and Knowlton 1998; Heil et al. 2009), because it underlies all types of host sanction mechanisms. Indeed, ant–plants adjust reward production to the presence and/or activity of the defending ants (Folgarait et al. 1994; Heil et al. 2000, 1997; Risch and Rickson 1981). In the present study we report several traits, most of which have been found to be phenotypically plastic, that likely contribute to the stabilisation of an obligate ant–plant mutualism against invasion by parasites and the evolution of cheaters. In some cases, the underlying chemical traits and signals could be identified successfully. For example, we found that the food rewards produced by Mesoamerican *Acacia* myrmecophytes are chemically adapted to the specific needs of the resident mutualist ants and at the same time chemically protected from exploiters, such as generalist ants and EFN-infesting microorganisms. The mutualist ant species involved were found to be specialised to feed exclusively on host-derived EFN and FBs, likely because this vegetarian diet is supplemented by nitrogen that is derived from bacterial symbionts. These specialisations might make the use of host-derived ant rewards by the mutualists more efficient as compared to the less specialised parasites and thus can contribute to the stabilisation of the association of host plants with mutualist ants. However, important physiological adaptations of the mutualist ants consisted in a loss of digestive capacities, making these ant species more dependent on the host-derived and chemically specialised food rewards. Because these ants, thus, depend on the state of their host plant, ‘cheating’ ants that show tendencies to cease the protection service would likely receive lower amounts of host-derived rewards than ‘honest’ mutualists. In summary, our studies revealed two mechanisms that contribute to the evolutionary stabilisation of this ant–plant mutualism against exploitation by parasites and the evolution of cheaters: (i) the production of chemically specialised rewards for the nutrition of physiologically adapted ant mutualists and (ii) reciprocal responses among phenotypically plastic partners.

Acknowledgments We thank Rosa Maria Adame-Álvarez, Ralf Krüger and Juan Carlos Silva Bueno for help with field work, Doyle McKey for many valuable comments on an earlier version of this manuscript and the German Research Foundation (DFG grant He3169/4-2) and CONACyT for financial support.

References

- Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. *Science* 294:321–326
- Baker HG, Baker I (1975) Studies of nectar-constitution and pollinator-plant coevolution. In: Gilbert F, Raven PH (eds)

- Coevolution of animals and plants. University of Texas Press, Austin, pp 100–140
- Baker HG, Opler PA, Baker I (1978) A comparison of the amino acid complements of floral and extrafloral nectars. *Bot Gaz* 139:322–332
- Beach RM, Todd JW, Baker SH (1985) Nectaried and nectariless cotton cultivars as nectar sources for the adult soybean looper. *J Entomol Sci* 20:233–236
- Bentley BL (1977) Extrafloral nectaries and protection by pugnacious bodyguards. *Annu Rev Ecol Syst* 8:407–427
- Bergstrom CT, Lachmann M (2003) The Red King effect: when the slowest runner wins the coevolutionary race. *Proc Natl Acad Sci USA* 100:593–598
- Blüthgen N, Fiedler K (2004) Preferences for sugars and amino acids and their conditionality in a diverse nectar-feeding ant community. *J Anim Ecol* 73:155–166
- Bové JL, Wäckers FL (2003) Gustatory perception and metabolic utilization of sugars by *Myrmica rubra* ant workers. *Oecologia* 136:508–514
- Bronstein JL (1994) Our current understanding of mutualism. *Q Rev Biol* 69:31–51
- Bronstein JL (2001) The exploitation of mutualisms. *Ecol Lett* 4:277–287. doi:10.1046/j.1461-0248.2001.00218.x
- Bronstein JL, Huxman T, Horvath B, Farabee M, Davidowitz G (2009) Reproductive biology of *Datura wrightii*: the benefits of a herbivorous pollinator. *Ann Bot* 103:1435–1443. doi:10.1093/aob/mcp053
- Brouat C, Garcia N, Andary C, McKey D (2001) Plant lock and ant key: pairwise coevolution of an exclusion filter in an ant–plant mutualism. *Proc R Soc Lond B* 268:2131–2141. doi:10.1098/rspb.2001.1763
- Brysch-Herzberg M (2005) Ecology of yeasts in plant–bumblebee mutualism in Central Europe. *FEMS Microbiol Ecol* 50:87–100
- Bshary R, Grutter AS (2005) Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biol Lett* 1:396–399
- Bull JJ, Rice WR (1991) Distinguishing mechanisms for the evolution of co-operation. *J Theor Biol* 149:63–74. doi:10.1016/S0022-5193(05)80072-4
- Carter C, Thornburg RW (2004) Is the nectar redox cycle a floral defense against microbial attack? *Trends Plant Sci* 9:320–324
- Chandler SM, Wilkinson TL, Douglas AE (2008) Impact of plant nutrients on the relationship between a herbivorous insect and its symbiotic bacteria. *Proc R Soc Lond B* 275:565–570. doi:10.1098/rspb.2007.1478
- Clement LW, Köppen S, Brand WA, Heil M (2008) Strategies of a parasite of the ant–*Acacia* mutualisms. *Behav Ecol Sociobiol* 26:953–962. doi:10.1007/s00265-007-0520-1
- Cornelius ML, Grace JK, Yates JR (1996) Acceptability of different sugars and oils to three tropical ant species (Hymen., Formicidae). *Anz Schädlingsk Pflanzensch Umweltsch* 69:41–43
- Dalecky A, Gaume L, Schatz B, McKey D, Kjellberg F (2005) Facultative polygyny in the plant–ant *Petalomyrmex phylax* (Hymenoptera : Formicinae): sociogenetic and ecological determinants of queen number. *Biol J Linn Soc* 86:133–151
- Davidson DW, Patrell-Kim L (1996) Tropical arboreal ants: why so abundant? In: Gibson AC (ed) Neotropical biodiversity and conservation. Mildred E. Mathias Botanical Garden, University of California, Los Angeles, pp 127–140
- Davidson DW, Cook SC, Snelling RR, Chua TH (2003) Explaining the abundance of ants in lowland tropical rainforest canopies. *Science* 300:969–972. doi:10.1126/science.1082074
- Dean MD (2006) A *Wolbachia*-associated fitness benefit depends on genetic background in *Drosophila simulans*. *Proc R Soc Lond B* 273:1415–1420. doi:10.1098/rspb.2005.3453
- Denison RF, Kiers ET (2004) Lifestyle alternatives for rhizobia: mutualism, parasitism, and forgoing symbiosis. *FEMS Microbiol Lett* 237:187–193
- Doebeli M, Hauert C (2005) Models of cooperation based on the Prisoner's Dilemma and the Snowdrift game. *Ecol Lett* 8:748–766. doi:10.1111/j.1461-0248.2005.00773.x
- Doebeli M, Knowlton N (1998) The evolution of interspecific mutualisms. *Proc Natl Acad Sci USA* 95:8676–8680
- Doebeli M, Hauert C, Killingback T (2004) The evolutionary origin of cooperators and defectors. *Science* 306:859–862
- Douglas AE (2008) Conflict, cheats and the persistence of symbioses. *New Phytol* 177:849–858
- Duarte Rocha CF, Godoy Bergallo H (1992) Bigger ant colonies reduce herbivory and herbivore residence time on leaves of an ant–plant: *Azteca muelleri* vs. *Coelomera ruficornis* on *Cecropia pachystachia*. *Oecologia* 91:249–252
- Edwards DP, Hassall M, Sutherland WJ, Yu DW (2006) Selection for protection in an ant–plant mutualism: host sanctions, host modularity and the principal-agent game. *Proc R Soc Lond B* 273:595–602. doi:10.1098/rspb.2005.3273
- Eilmus S, Heil M (2009) Bacterial associates of arboreal ants and their putative functions in an obligate ant–plant mutualism. *Appl Environ Microbiol* 75:4324–4332. doi:10.1128/AEM.00455-09
- Federle W, Fiala B, Zizka G, Maschwitz U (2001) Incident daylight as orientation cue for hole-boring ants: prostomata in *Macaranga* ant–plants. *Ins Soc* 48:165–177
- Feldhaar H, Fiala B, bin Hashim R, Maschwitz U (2000) Maintaining an ant–plant symbiosis: secondary polygyny in the *Macaranga triloba*-*Crematogaster* sp. association. *Naturwissenschaften* 87:408–411
- Ferrière R, Gauduchon M, Bronstein JL (2007) Evolution and persistence of obligate mutualists and exploiters: competition for partners and evolutionary immunization. *Ecol Lett* 10:115–126. doi:10.1111/j.1461-0248.2006.01008.x
- Folgarait PJ, Johnson HL, Davidson DW (1994) Responses of *Cecropia* to experimental removal of Müllerian bodies. *Funct Ecol* 8:22–28
- Fonseca CR (1993) Nesting space limits colony size of the plant–ant *Pseudomyrmex concolor*. *Oikos* 67:473–482
- Foster KR, Wenseleers T (2006) A general model for the evolution of mutualisms. *J Evol Biol* 19:1283–1293
- Gaume L, McKey D (1999) An ant–plant mutualism and its host-specific parasite: activity rhythms, young leaf patrolling, and effects on herbivores of two specialist plant–ants inhabiting the same myrmecophyte. *Oikos* 84:130–144
- Gaume L, Zacharias M, Borges RM (2005) Ant–plant conflicts and a novel case of castration parasitism in a myrmecophyte. *Evol Ecol Res* 7:435–452
- González-Teuber M, Heil M (2009) The role of extrafloral nectar amino acids for the preferences of facultative and obligate ant mutualists. *J Chem Ecol* 35:459–468. doi:10.1007/s10886-009-9618-4
- González-Teuber M, Eilmus S, Muck A, Svatos A, Heil M (2009) Pathogenesis-related proteins protect extrafloral nectar from microbial infestation. *Plant J* 58:464–473. doi:10.1111/j.1365-3113.2009.03790.x
- Hamilton WD (1964) The genetical evolution of social behaviour I and II. *J Theor Biol* 7:1–16, 17–52
- Heil M (2008) Indirect defence via tritrophic interactions. *New Phytol* 178:41–61. doi:10.1111/j.1469-8137.2007.02330.x
- Heil M, McKey D (2003) Protective ant–plant interactions as model systems in ecological and evolutionary research. *Annu Rev Ecol Syst* 34:425–453. doi:10.1146/annurev.ecolsys.34.011802.132410
- Heil M, Fiala B, Linsenmair KE, Zotz G, Menke P, Maschwitz U (1997) Food body production in *Macaranga triloba*

- (Euphorbiaceae): a plant investment in anti-herbivore defence via mutualistic ant partners. *J Ecol* 85:847–861
- Heil M, Fiala B, Baumann B, Linsenmair KE (2000) Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Funct Ecol* 14:749–757. doi:10.1046/j.1365-2435.2000.00480.x
- Heil M, Hilpert A, Fiala B, Linsenmair KE (2001a) Nutrient availability and indirect (biotic) defence in a Malaysian ant-plant. *Oecologia* 126:404–408. doi:10.1007/s004420000534
- Heil M, Koch T, Hilpert A, Fiala B, Boland W, Linsenmair KE (2001b) Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proc Natl Acad Sci USA* 98:1083–1088
- Heil M, Baumann B, Krüger R, Linsenmair KE (2004a) Main nutrient compounds in food bodies of Mexican *Acacia* ant-plants. *Chemoecology* 14:45–52. doi:10.1007/s00049-003-0257-x
- Heil M, Greiner S, Meimberg H, Krüger R, Noyer J-L, Heubl G, Linsenmair KE, Boland W (2004b) Evolutionary change from induced to constitutive expression of an indirect plant resistance. *Nature* 430:205–208. doi:10.1038/nature02703
- Heil M, Hilpert A, Krüger R, Linsenmair KE (2004c) Competition among visitors to extrafloral nectaries as a source of ecological costs of an indirect defence. *J Trop Ecol* 20:201–208. doi:10.1017/S026646740300110X
- Heil M, Rattke J, Boland W (2005) Post-secretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* 308:560–563. doi:10.1126/science.1107536
- Heil M, González-Teuber M, Clement LW, Kautz S, Verhaagh M, Silva Bueno JC (2009) Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters. *Proc Natl Acad Sci USA* 106:18091–18096. doi:10.1073/pnas.0904304106
- Herrera CM, Garcia IM, Perez R (2008) Invisible floral larcenies: microbial communities degrade floral nectar of bumble bee-pollinated plants. *Ecology* 89:2369–2376
- Herrera CM, De Vega C, Canto A, Pozo MI (2009) Yeasts in floral nectar: a quantitative survey. *Ann Bot* 103:1415–1423. doi:10.1093/aob/mcp026
- Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH (2008) How many species are infected with *Wolbachia*?—a statistical analysis of current data. *FEMS Microbiol Lett* 281:215–220. doi:10.1111/j.1574-6968.2008.01110.x
- Hoeksema JD, Bruna EM (2000) Pursuing the big questions about interspecific mutualism: a review of theoretical approaches. *Oecologia* 125:321–330. doi:10.1007/s004420000496
- Hoeksema JD, Kummel M (2003) Ecological persistence of the plant-mycorrhizal mutualism: a hypothesis from species coexistence theory. *Am Nat* 162:S40–S50. doi:10.1086/378644
- Hölldobler B, Wilson EO (1990) *The ants*. Springer, Berlin
- Izzo TJ, Vasconcelos HL (2002) Cheating the cheater: domatia loss minimizes the effects of ant castration in an Amazonian ant-plant. *Oecologia* 133:200–205. doi:10.1007/s00442-002-1027-0
- Janzen DH (1966) Coevolution of mutualism between ants and acacias in Central America. *Evolution* 20:249–275
- Janzen DH (1974) *Swollen-thorn acacias of Central America*. Smithsonian Institution Press, Washington
- Janzen DH (1975) *Pseudomyrmex nigropilosa*: a parasite of a mutualism. *Science* 188:936–937. doi:10.1126/science.188.4191.936
- Kautz S, Lumbsch HT, Ward PS, Heil M (2009a) How to prevent cheating: a digestive specialization ties mutualistic plant–ants to their ant–plant partners. *Evolution* 63:839–853. doi:10.1111/j.1558-5646.2008.00594.x
- Kautz S, Pauls SU, Ballhorn DJ, Heil M (2009b) Polygynous supercolonies of the acacia–ant *Pseudomyrmex peperi*, an inferior colony founder. *Mol Ecol*. doi:10.1111/j.1365-294X.2009.04395.x
- Kautz S, Schmid VS, Trindl A, Heinze J, Ballhorn DJ, Heil M (2009c) Isolation and characterization of microsatellite loci in the plant–ant *Pseudomyrmex ferrugineus* (Formicidae: Pseudomyrmecinae) and cross-testing for two congeneric species. *Mol Ecol Resources* 9:1016–1019. doi:10.1111/j.1755-0998.2009.02569.x
- Kiers ET, Rousseau RA, West SA, Denison RF (2003) Host sanctions and the legume–rhizobium mutualism. *Nature* 425:78–81. doi:10.1038/nature01931
- Kobayashi S, Asai T, Fujimoto Y, Kohshima S (2008) Anti-herbivore structures of *Paulownia tomentosa*: morphology, distribution, chemical constituents and changes during shoot and leaf development. *Ann Bot* 101:1035–1047. doi:10.1093/aob/mcn033
- Koptur S (1992) Extrafloral nectary-mediated interactions between insects and plants. In: Bernays EA (ed) *Insect–plant interactions*, vol IV. CRC Press, Boca Raton, pp 81–129
- Lanza J, Vargo EL, Pulim S, Chang YZ (1993) Preferences of the fire ants *Solenopsis invicta* and *S. geminata* (Hymenoptera: Formicidae) for amino acid and sugar components of extrafloral nectars. *Environ Entomol* 22:411–417
- Letourneau DK (1990) Code of ant–plant mutualism broken by parasite. *Science* 248:215–217. doi:10.1126/science.248.4952.215
- Limpens E, Bisseling T (2003) Signaling in symbiosis. *Curr Opin Plant Biol* 6:343–350
- Mithöfer A (2002) Suppression of plant defence in rhizobia–legume symbiosis. *Trends Plant Sci* 10:440–444
- Mody K, Linsenmair KE (2004) Plant-attracted ants affect arthropod community structure but not necessarily herbivory. *Ecol Entomol* 29:217–225
- Mondor EB, Addicott JF (2003) Conspicuous extra-floral nectaries are inducible in *Vicia faba*. *Ecol Lett* 6:495–497
- Morris WF, Bronstein JL, Wilson WG (2003) Three-way coexistence in obligate mutualist-exploiter interactions: the potential role of competition. *Am Nat* 161:860–875
- Nardi JB, Mackie RI, Dawson JO (2002) Could microbial symbionts of arthropod guts contribute significantly to nitrogen fixation in terrestrial ecosystems? *J Insect Physiol* 48:751–763
- Ness JH, Morris WF, Bronstein JL (2006) Integrating quality and quantity of mutualistic service to contrast ant species protecting *Ferocactus wislizeni*. *Ecology* 87:912–921
- Nicolson SW, Thornburg RW (2007) Nectar chemistry. In: Nicolson SW, Nepi M, Pacini E (eds) *Nectaries and nectar*. Springer, Heidelberg, pp 215–263
- Palmer TM (2004) Wars of attrition: colony size determines competitive outcomes in a guild of African acacia ants. *Anim Behav* 68:993–1004
- Pellmyr O, Huth CJ (1994) Evolutionary stability of mutualism between yuccas and yucca moth. *Nature* 372:257–260
- Perlman SJ, Hunter MS, Zchori-Fein E (2006) The emerging diversity of *Rickettsia*. *Proc R Soc Lond B* 273:2097–2106. doi:10.1098/rspb.2006.3541
- Pulice CE, Packer AA (2008) Simulated herbivory induces extrafloral nectary production in *Prunus avium*. *Funct Ecol* 22:801–807. doi:10.1111/j.1365-2435.2008.01440.x
- Raine NE, Gammans N, Macfadyen IJ, Scrivner GK, Stone GN (2004) Guards and thieves: antagonistic interactions between two ant species coexisting on the same ant–plant. *Ecol Entomol* 29:345–352. doi:10.1111/j.0307-6946.2004.00608.x
- Rickson FR (1969) Developmental aspects of the shoot apex, leaf, and Beltian bodies of *Acacia cornigera*. *Am J Bot* 56:195–200
- Rickson FR (1975) The ultrastructure of *Acacia cornigera* L. Beltian body tissue. *Am J Bot* 62:913–922
- Risch SJ, Rickson F (1981) Mutualism in which ants must be present before plants produce food bodies. *Nature* 291:149–150. doi:10.1038/291149a0
- Roesch C, Bothe H (2005) Improved assessment of denitrifying, nitrogen-fixing, and total-community bacteria by terminal

- restriction fragment length polymorphism [tRFLP] analysis using multiple restriction enzymes. *Appl Environ Microbiol* 71:2026–2035
- Rogers WE, Siemann E, Lankau RA (2003) Damage induced production of extrafloral nectaries in native and invasive seedlings of Chinese tallow tree (*Sapium sebiferum*). *Am Midl Natural* 149:413–417
- Sachs JL, Simms EL (2006) Pathways to mutualism breakdown. *Trends Ecol Evol* 21:585–592. doi:10.1016/j.tree.2006.06.018
- Sachs JL, Mueller UG, Wilcox TP, Bull JJ (2004) The evolution of cooperation. *Q Rev Biol* 79:135–160
- Sandhu DK, Waraich MK (1985) Yeast associated with pollinating bees and flower nectar. *Microbial Ecol* 11:51–58
- Segraves K, Althoff D, Pellmyr O (2005) Limiting cheaters in mutualism: evidence from hybridization between mutualist and cheater yucca moths. *Proc R Soc Lond B* 272:2195–2201
- Simms EL, Taylor DL, Povich J, Shefferson RP, Sachs JL, Urbina M, Tausczik Y (2006) An empirical test of partner choice mechanisms in a wild legume–rhizobium interaction. *Proc R Soc Lond B* 273:77–81
- Stapel JO, Cortesero AM, DeMoraes CM, Tumlinson JH, Lewis WJ (1997) Extrafloral nectar, honeydew, and sucrose effects on searching behavior and efficiency of *Microplitis croceipes* (Hymenoptera: Braconidae) in cotton. *Environ Entomol* 26:617–623
- Stoll S, Gadau J, Gross R, Feldhaar H (2007) Bacterial microbiota associated with ants of the genus *Tetraponera*. *Biol J Linn Soc* 90:399–412. doi:10.1111/j.1095-8312.2006.00730.x
- Sultan SE (2000) Phenotypic plasticity for plant development, function and life history. *Trends Plant Sci* 5:537–542
- Thornburg RW (2007) Molecular biology of the *Nicotiana* floral nectary. In: Nicolson SW, Nepi M, Pacini E (eds) *Nectaries and Nectar*. Springer, Heidelberg, pp 265–287
- Thornburg RW, Carter C, Powell A, Mittler R, Rizhsky L, Horner HT (2003) A major function of the tobacco floral nectary is defense against microbial attack. *Plant Syst Evol* 238:211–218
- Tillberg CV (2004) Friend or foe? A behavioral and stable isotopic investigation of an ant–plant symbiosis. *Oecologia* 140:506–515. doi:10.1007/s00442-004-1601-8
- Tobin JE (1995) Ecology and diversity of tropical forest canopy ants. In: Lowman MD, Nadkarni NM (eds) *Forest canopies*. Academic Press, San Diego, pp 129–147
- Van Loon LC, Van Strien EA (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol Mol Plant Pathol* 55:85–97
- Van Loon LC, Rep M, Pieterse CMJ (2006) Significance of inducible defense-related proteins in infected plants. *Annu Rev Phytopathol* 44:135–162
- Vander Meer RK, Lofgren CS, Seawright JA (1995) Specificity of the red imported fire ant (Hymenoptera, Formicidae) phagostimulant response to carbohydrates. *Florida Entomol* 78:144–154
- Wäckers FL, Wunderlin R (1999) Induction of cotton extrafloral nectar production in response to herbivory does not require a herbivore-specific elicitor. *Entomol Exp Appl* 91:149–154
- Ward PS (1993) Systematic studies on *Pseudomyrmex* acacia–ants (Hymenoptera: Formicidae: Pseudomyrmecinae). *J Hym Res* 2:117–168
- Werren JH, Windsor D, Guo LR (1995) Distribution of *Wolbachia* among neotropical arthropods. *Proc R Soc Lond B* 262:197–204
- West SA, Kiers ET, Simms EL, Denison RF (2002) Sanctions and mutualism stability: why do rhizobia fix nitrogen? *Proc R Soc Lond B Biol Sci* 269:685–694
- Wooley SC, Donaldson JR, Gusse AC, Lindroth RL, Stevens MT (2007) Extrafloral nectaries in aspen (*Populus tremuloides*): Heritable genetic variation and herbivore-induced expression. *Ann Bot* 100:1337–1346. doi:10.1093/aob/mcm220
- Yu DW (2001) Parasites of mutualisms. *Biol J Linn Soc* 72:529–546
- Yu DW, Pierce NE (1998) A castration parasite of an ant–plant mutualism. *Proc R Soc Lond B* 265:375–382. doi:10.1098/rspb.1998.0305
- Zientz E, Beyaert N, Gross R, Feldhaar H (2006) Relevance of the endosymbiosis of *Blochmannia floridanus* and carpenter ants at different stages of the life cycle of the host. *Schriftenr Vegetationskunde* 72:6027–6033