

## Stabilizing Mutualisms Threatened by Exploiters: New Insights from Ant–Plant Research

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### ABSTRACT

Mutualisms are commonly threatened by parasites and cheaters: species that exploit the host-derived resources without providing an adequate service. Here, we summarize mechanisms for the stabilization of obligate defensive ant–plant mutualisms, a typical element of tropical lowland forests. Host plants exert partner choice and can sanction non-defending ants by shedding the domatia that serve as nesting space or ceasing the production of ant rewards. Hosts can also restrict the exploitation of the ant rewards by means of specific biochemical traits that decrease their quality for non-adapted generalist exploiters and, thus, convert them into exclusive rewards. Reward provisioning can even shift the competitive balance between mutualists and exploiters in favor of the mutualists. In turn, plant-ants show adaptations in their colony structure and changes in their digestive capacities that enhance their efficiency in the use of the host-derived resources. Founding queens use plant odors for host choice behavior, and ants not supplied with adequate amounts of EFN decrease their defensive service and thereby exert partner sanctions. Theoretical models and empirical research into mutualisms usually focus on actions that are taken by the host. Using ant-plants as model systems, we are now discovering the importance of contributions that come from the symbiont. This discovery indicates the potential for multiple reciprocal interactions between phenotypically plastic hosts and symbionts, which contribute significantly to what is still considered a miracle: the stability of mutualisms in the presence of exploiters.

*Key words:* exclusive rewards; host choice; host sanctions; myrmecophyte; partner choice; partner sanctions.

ANT–PLANT INTERACTIONS REPRESENT A TYPICAL ELEMENT IN TROPICAL FORESTS. Facultative interactions between ants and plants exist in virtually all terrestrial ecosystems besides arctic regions and high mountains, but obligate interactions between so-called myrmecophytes and their (usually highly specialized) ant inhabitants are restricted to the tropics (Heil & McKey 2003). The obligate defensive mutualisms on which we focus here comprise host plants that provide nesting space (in hollow structures termed domatia) and usually also produce extrafloral nectar (EFN) or cellular food bodies (FBs) as food rewards. In some systems, scale insects form part of the mutualistic system and contribute to the nutrition of the ants (Janzen 1966, Heckroth *et al.* 1998, Gaume *et al.* 2000, Defosse *et al.* 2009). In turn, the ants protect their host from herbivores, pathogens, and encroaching vegetation. These myrmecophytes commonly are pioneer trees that can completely dominate secondary forests, likely because of the highly efficient defence against biotic stress that resident ant colonies provide to their hosts (Heil 2008, Chamberlain & Holland 2009, Rosumek *et al.* 2009, Koricheva & Romero 2012).

Like all mutualisms, ant–plant mutualisms are prone to exploitation by non-reciprocating exploiters, *i.e.*, species that inhabit the host and utilize the resources that serve to reward the

symbiont, however, without paying for the service (Bronstein 2001, Yu 2001). Exploiters save the time and energy that mutualists spend on reciprocating. Therefore, many authors consider them as competitively superior, at least as long as resource availability remains unchanged. Exploiters can evolve from former mutualists that cease service provisioning and then represent cheaters, or they can invade the mutualism starting from an originally independent lifestyle and then represent parasites of the mutualism (Bronstein 2001, Segraves *et al.* 2005, Kautz *et al.* 2009a). Exploitation has been reported for a wide range of mutualisms, including nectar robbing (*e.g.*, bees and birds) (Roubik 1982, Maloof & Inouye 2000), domatia exploiters (Gaume *et al.* 2006, Shenoy & Borges 2008), mycorrhizal fungi that uptake the plant carbon but transfer no nutrients to the plant (Smith *et al.* 1996), and strains of *Rhizobium* and *Bradyrhizobium* that transfer less or no nitrogen to the host than mutualistic strains (Wilkinson *et al.* 1996). In defensive ant–plant mutualisms, *Phyllobaenus* beetles exploit the shelter and food rewards produced by *Piper* plants (Letourneau 1983), a foraging spider (*Bagheera kiplingi*) that lives in the hollow spines of Mexican acacias uses plant-derived food body rewards for its nutrition (Meehan *et al.* 2009), several ants inhabit the plant without providing a specific defensive service (Janzen 1975, Raine *et al.* 2004, Clement *et al.* 2008, see Fig. 1), and even ants that efficiently fend off herbivores can damage their host by manipulating its reproductive efforts for their own

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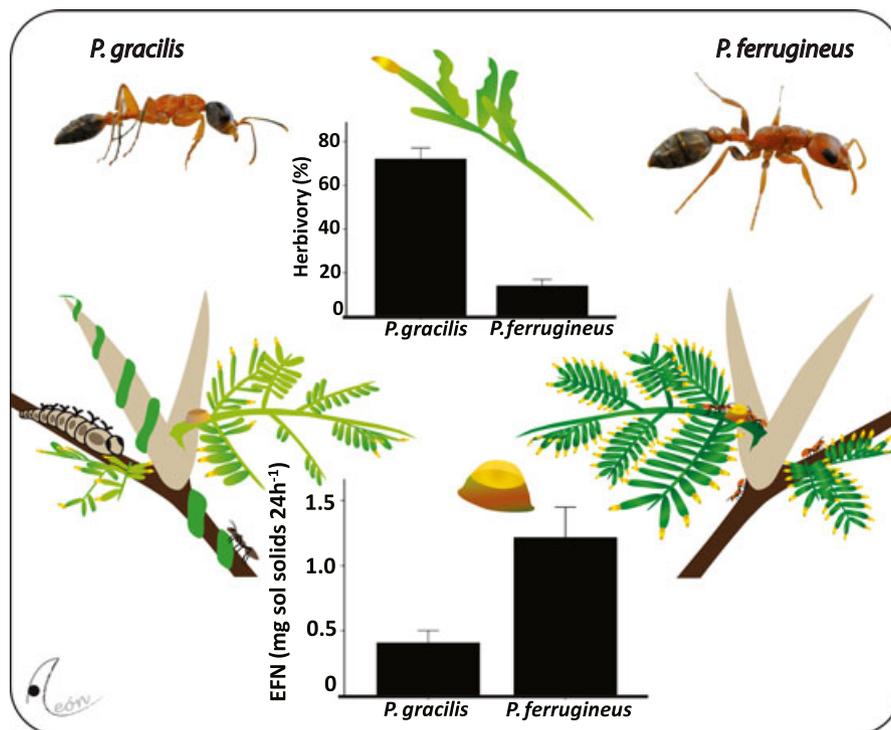


FIGURE 1. An exploiter ant and its effect on the host plant: Mesoamerican ant acacias can be inhabited by exploiter ants (*Pseudomyrmex gracilis*, left panel) which lack a specific defensive behavior and therefore cause high rates of leaf damage. By contrast, mutualistic *Pseudomyrmex ferrugineus* ants protect their host against herbivory. Both ant species feed on food bodies (FBs) and extrafloral nectar (EFN), although plants inhabited by the exploiter, *P. gracilis*, appear to reduce reward production and thereby exert host sanctions. Host sanctions by bull's horn acacias: Mesoamerican ant acacias reduce the production of ant rewards (extrafloral nectar, EFN: mean secretion as milligrams [mg] of soluble [sol] solids) when being inhabited by the parasitic ant, *P. gracilis* (left panel), whereas high amounts of rewards are provided to the mutualist, *P. ferrugineus* (right panel) (Clement *et al.* 2008, Heil *et al.* 2009).

benefits (Yu & Pierce 1998, Izzo & Vasconcelos 2002, Gaume *et al.* 2005a).

Research into the stability of mutualisms has traditionally focused on pollination and the root – Rhizobia and root – mycorrhiza mutualism. However, ant–plant interactions are increasingly being used to study the underlying mechanisms (Bronstein 1998, Heil & McKey 2003). In this review, we first give a short introduction to the different mechanisms that have been suggested for the stabilization of mutualisms, then present exploiters that are commonly reported for ant–plant mutualisms and lastly discuss in detail which stabilizing mechanisms have been rejected, corrected, confirmed, or even discovered, in the research into ant–plant mutualisms. We conclude with a short outlook discussing how likely the mechanisms that have been described for ant–plant interactions are to stabilize also other, non-ant-plant and non-tropical mutualisms.

## CONCEPTS IN THE STABILIZATION OF MUTUALISMS

Theoretical research distinguishes among five main strategies by which mutualisms can be stabilized against exploitation. Unfortunately, different authors make different assumptions and different

use of central terms, for which reason these concepts are under heavy and controversial discussion (Weyl *et al.* 2010, 2011, Kiers *et al.* 2011a). In the following, we shortly introduce the most commonly reported stabilizing mechanisms as they have been described for other mutualisms and define the terms as we will use them in the present article.

## LONG-SPURRED ORCHIDS AND EXCLUSIVE REWARDS

Mechanical barriers can represent the primary means to restrict access to a reward and protect it from exploitation. The long nectary spurs of *Angraecum sesquipedale*, a Malagasy orchid, make floral nectar inaccessible to most insects and thus turn it into an exclusive reward for a specialized, long-tongued hawkmoth (Darwin 1862), thereby increasing the probability of pollen transfer (Rodríguez-Gironés & Santamaría 2007). This orchid represents a classical example of the outcome of a co-evolutionary process (Martins & Johnson 2007). Subsequent studies confirmed the importance of anatomical structures such as long corolla tubes or nectar spurs for the effective protection of floral nectar from nectar robbers (Micheneau *et al.* 2009, Sletvold & Ågren 2010).

## PARTNER CHOICE

Partner choice refers to the selection of suitable future partners before the symbiosis is being established and is usually based on specific characteristics of the potential symbiont (Bull & Rice 1991). Partner choice allows host individuals to differentially reward cooperative versus uncooperative partners in advance of any possible exploitation (Bull & Rice 1991). This phenomenon has been studied particularly well for the root–Rhizobia mutualism, in which Rhizobia strains must identify themselves as potential mutualists via the secretion of specific chemical signals (Nod factors) (Oldroyd 2001, Simms *et al.* 2006, Oldroyd & Downie 2008). The importance of partner choice mechanisms, however, has also been demonstrated for other mutualism, *e.g.*, for the cleaner fish–host interaction (Bshary & Grutter 2002), plant–pollinator (Bull & Rice 1991), plant–mycorrhiza (Kiers *et al.* 2011b), and the ant–fungus interaction (Mueller *et al.* 2004, Zhang *et al.* 2007).

## HOST SANCTIONS

Host sanctions apply when the symbiosis has already been established. Here, the host monitors the action of its symbiont to punish exploiters, usually via a reduction in reward provisioning. For example, plant roots cease the allocation of assimilates toward nodules formed by non N-fixing bacteria or mycorrhizal fungi that turn pathogenic (West *et al.* 2002, Kiers *et al.* 2003, 2011b, Kiers & Denison 2008). A recent study demonstrated that host sanctions can even function when one host is interacting with multiple symbionts of different quality, which demonstrates an impressive level of specificity of this strategy, at least in the fig–fig wasp mutualism (Jandér *et al.* 2012).

## PARTNER FIDELITY FEEDBACK

The concept of mutualism is based on the major idea that each partner benefits from the interaction with the other partner. The benefits provided by partner 1 to partner 2 automatically feed back to partner 1 always when the quantity or quality of the resources provided to partner 1 depend on the performance or vigor of partner 2 (Connor 1986, Sachs *et al.* 2004). Therefore, several authors assume that natural selection favors mutualists over cheaters simply because partner fidelity feedback inevitable reduced the fitness of cheaters (Weyl *et al.* 2010).

## COMPETITIVE SCREENING

Partner choice as well as host sanctions require that hosts can evaluate the identity or the actions of their symbionts, whereas partner fidelity feedback occurs as an inevitable consequence of the negative effects that the failure to reciprocate has on the other partner. Recently, partner screening has been proposed as an alternative mechanism for the stabilization of mutualisms (Archetti *et al.* 2011a,b). Partner screening means that the host imposes a ‘contract’ that consists of appropriate costs and

benefits of being a mutualist. The decision of the potential symbiont whether or not to make this investment depends on the net benefits that it can gain in the mutualism, thereby causing the potential symbionts to screen themselves according to their quality as mutualists. Only the adapted mutualists might gain sufficient benefit from engaging in the interaction to make it attractive for them to pay the cost of entry. This model does not require hosts to have any direct information on the quality of the symbionts.

## EXPLOITERS OF ANT–PLANT MUTUALISMS

Ant–plant mutualisms are transmitted horizontally, usually comprise more than one species of both host and symbiont and depend commonly on rewards that are presented openly on the plant surface. All these characteristics make ant–plant mutualisms particularly prone to invasions by taxonomically and functionally different exploiters. In this respect, ant–plant mutualisms resemble the general case, because the vast majority of exploiters are generalist foragers, such as pollen and nectar robbers, which transiently feed on rewards when these are available (Addicott 1986, West *et al.* 1996, Jackson 2009). In the following sections, we shortly summarize the types of exploiters that most commonly have been reported for ant–plant mutualisms.

## NON-DEFENDING ANTS

The most important service that plant–ants provide to their hosts is the defence against biotic stressors, such as herbivores and pathogens. Facultative as well as obligate ant–plant mutualisms can include more than one ant species, and ants vary in their patrolling behavior and defensive efficiency (Fiala *et al.* 1994, Hossaert-McKey *et al.* 2001, Palmer *et al.* 2002, 2003, Bruna *et al.* 2004, Debout *et al.* 2005, Frederickson 2005, Gaume *et al.* 2005b, 2006, Xu & Chen 2010). Although under certain circumstances it can be beneficial for the plant to associate with a variety of different species of ants (Labeyrie *et al.* 2001, Palmer *et al.* 2010), the differences in their defensive quality make some ants more desirable symbionts than others. Species such as *Camponotus planatus* (Raine *et al.* 2004), *Pseudomyrmex nigropilosus*, and *Pseudomyrmex gracilis* (Janzen 1975, Clement *et al.* 2008) inhabit *Acacia* myrmecophytes without providing them with an efficient protection (Fig. 1), *Catantlus mckeyi* exploits *Leonardoxa africana* (Gaume & McKey 1999), and several species of *Cephalotes* and *Crematogaster* act as non-defending parasites of *Cordia alliodora* (Tillberg 2004). However, most ants are foragers and protect their food sources, and particularly the surroundings of their nest, from potential competitors or invaders. Thus, the presence of any type of ant colony on a plant will usually cause some protective effect, compared with the completely ant-free plant. It therefore remains an open question whether these ants represent true exploiters or should better be understood as ‘less efficient mutualists’. Still the parasitic ants can replace the good defenders under certain circumstances (Heil 2013) and their presence reduces the probability

at which a host will be colonized by a high-quality mutualist (Clement *et al.* 2008, Heil *et al.* 2009). We argue, therefore, that non-defending ants are correctly understood as exploiters of obligate ant–plant mutualisms.

## CASTRATION PARASITES

In some ant–plant interactions, the ant castrates the host, meaning that it physically removes the reproductive organs (Baudoin 1974, Frederickson 2005). Although final experimental evidence is missing, this behavior is commonly interpreted as shifting the allocation patterns in the plant from reproductive to vegetative growth and, thus, to the production of more ant rewards. For example, workers of *Allomerus octoarticulatus* protect the new leaves of *Cordia nodosa* from herbivory, which directly benefits the ants themselves because they live in stem domatia, one of which is produced with every new shoot. The ants, however, destroy flowers and thereby in most cases reduce the reproduction of their host (Edwards & Yu 2008, Szilágyi *et al.* 2009). Moreover, since *Allomerus* prevents their host from associating with the good mutualist, ants of the genus *Azteca*, its presence imposes a high cost to the host and *Allomerus* clearly represents a parasite of the *Cordia–Azteca* mutualism (Yu & Pierce 1998, Frederickson 2009). Interestingly, the same ant species (*A. octoarticulatus*) has been reported as an obligate mutualist of *Hirtella* plants, which use the abortion of leaves and domatia as strategy of development: this species apparently employs host sanctions to prevent its inhabitant from cheating (Izzo & Vasconcelos 2002). In fact, ants due to their general aggressive behavior will usually reduce pollinator visits to the flowers of their host plants, for which reason it appears likely that all ant-defended plants require strategies to control ant–pollinator conflicts (Willmer & Stone 1997, Adler 2000, Ghazoul 2001, Raine *et al.* 2002, Gaume *et al.* 2005a, Hernández-Cumplido *et al.* 2010, Heil 2011, Holland *et al.* 2011).

## EXPLOITERS OF FOOD REWARDS

Most myrmecophytes provide their ants with FBs, EFN, or both. FBs contain lipids and proteins and both types of ant rewards are rich in carbohydrates, thus appearing to be adapted to specifically serve as the major or exclusive food source for animals (Heil *et al.* 1998, Fischer *et al.* 2002). Thus, ant rewards represent highly attractive targets for all types of exploiters: animals that simply use these rewards for their own nutrition, without interacting in any other way with the plant. Whereas ‘non-defending ants’ might provide their host plant with at least some protective effect, these exploiters simply reduce the amount of rewards that are available to the mutualist ants. A classic example in this category is a species of *Phyllobaenus* beetles that prey on ant inhabitants of *Piper* plants and also feed on the FBs that are produced by these plants, even being capable of chemically inducing the production of this reward (Letourneau 1990). Similarly, the spider, *B. kiplingi*, preys on *Pseudomyrmex* ants that inhabit *Acacia* myrmecophytes and also feeds on the FBs (Jackson 2009, Mee-

han *et al.* 2009). Moreover, bees and wasps might act as robbers of EFN (González-Teuber *et al.* 2012).

Less conspicuous but perhaps quantitatively more important are microorganisms: yeasts and bacteria that infest EFN (González-Teuber & Heil 2009). Yeasts are commonly reported for floral nectars, which they use as a growing medium and chemically alter due to their own metabolism (Canto *et al.* 2008, Herrera *et al.* 2009, 2010, Canto & Herrera 2012). Therefore, many nectars contain specific hydrolytic proteins to prevent them from microbial infestation (Carter & Thornburg 2000, 2004, Thornburg *et al.* 2003). The recent discovery of chitinases and glucanases in the EFN of *Acacia* myrmecophytes (González-Teuber *et al.* 2009, 2010) indicates that EFN-infecting microorganisms might also represent common exploiters of ant–plant mutualisms.

## PROTECTING ANT–PLANT MUTUALISMS FROM EXPLOITERS

Myrmecophytes provide their ants with exclusive rewards. Mutualistic ants show multiple adaptations allowing them to more successfully compete for, and ultimately dominate, their host plants. The pruning of lianas and encroaching vegetation provides us with a nice example of how the symbiont can help to make its rewards exclusive. *Pseudomyrmex dendroicus*, a mutualist of *Triplaris americana* plants, prunes the shoots and petioles of plants in contact with its host and thereby reduces the danger of invasion by *Crematogaster* exploiters (Davidson *et al.* 1988). Similarly, *Crematogaster* (*Decacrema*) ants living on waxy *Macaranga* ant-plants prune more intensively when they live on a less waxy species on which most ants could walk easily (Federle *et al.* 2002).

Exclusive rewards, however, usually represent an adaptation of the host. The workers of the exploiter ant, *C. planatus*, which inhabits the thorns of *Acacia mayana* and feeds on its EFN, do not harvest the FBs (Raine *et al.* 2004). This example nicely illustrates that, in spite of their high nutrient contents, ant rewards are not invariably suitable for specialized mutualists and generalist exploiters. In fact, FBs of mesoamerican ant acacias contain protease inhibitors (PIs) (Wielsch *et al.* 2011) that successfully inhibit protease activity in the digestive tracts of seed-feeding beetles and likely other non-specialist exploiters (Fig. 2). By contrast, the proteases of the mutualist, *Pseudomyrmex ferrugineus*, appeared insensitive to this inhibitory activity (Orona-Tamayo *et al.* 2013a). As mentioned above, the same *Acacia* species protect their EFN from nectar-infecting microorganisms with pathogenesis-related proteins, such as glucanases and chitinases (González-Teuber *et al.* 2009, 2010). This EFN is further protected by a secreted invertase that keeps it free of sucrose and, thus, unattractive for local generalist ants (Heil *et al.* 2005). The mutualist ant, in turn, lacks invertase in the adult stage (Kautz *et al.* 2009a) and thus prefers sucrose-free EFN in direct choice tests (Heil *et al.* 2005). Finally, the ant acacias secrete the EFN only during a few hours per day, which facilitates its protection from exploiters (Raine *et al.* 2002, González-Teuber *et al.* 2012). These short peaks are

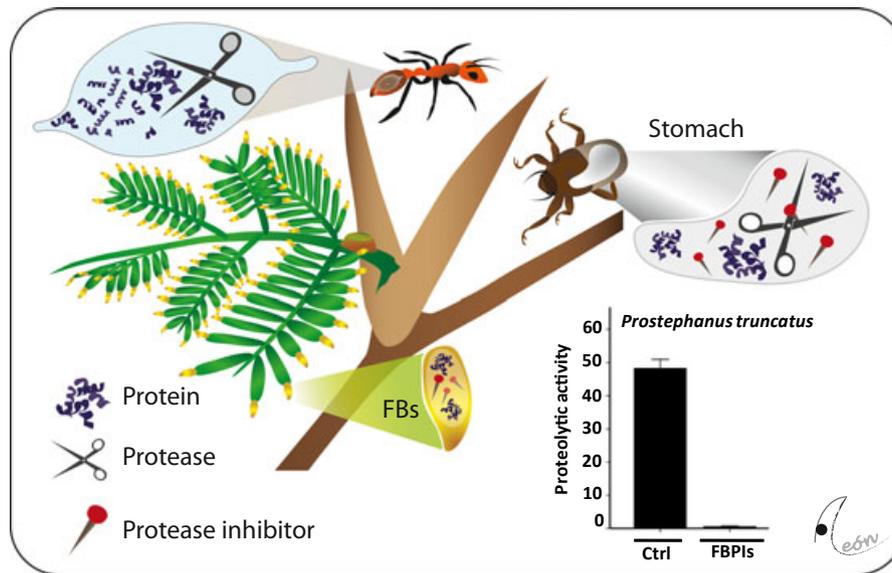


FIGURE 2. *Acacia* food bodies are exclusive rewards: The food bodies (FBs) produced by Mesoamerican ant-acacias contain protease inhibitors (PIs) that reduce proteolytic activity (graphical inset; Ctrl: control; FBPIs: Food Body Protease Inhibitors) in the digestive tracts of non-adapted potential exploiters whereas they do not affect the digestive capacities of the mutualist ant, *Pseudomyrmex ferrugineus* (Wielsch *et al.* 2011, Orona-Tamayo *et al.* 2013a).

physiologically achieved by the concurrent synthesis of the entire enzymatic machinery for nectar synthesis and secretion, directly before and during the hours of active secretion (Orona-Tamayo *et al.* 2013b). In summary, the rewards produced by mesoamerican ant acacias show multiple chemical and physiological characteristics that make them an almost exclusive food source for the resident mutualists, although the existence of exploiter ants and FB-robbing spiders shows that no protection is perfect. Unfortunately, comparable research into the detailed composition of other ant rewards is lacking, which makes it impossible to decide whether exclusive rewards represent a general characteristic of myrmecophytes. Phenomena such as toxic floral nectars (Adler 2000, Adler & Irwin 2005) and specific ant-attractants in elaiosomes (Brew *et al.* 1989, Hughes *et al.* 1994, Fischer *et al.* 2008, Boieiro *et al.* 2012), however, make it tempting to assume that all ant rewards possess a chemical composition that enhances their level of exclusiveness.

### PARTNER CHOICE AND HOST CHOICE IN THE ESTABLISHMENT OF ANT–PLANT MUTUALISMS

Choosing the right partner represents a crucial step in the establishment of all mutualisms. Evidently, both partners have an interest in taking this decision correctly. Morphological structures such as self-opening entrances to domatia occur in some myrmecophytes (Brouat *et al.* 2001, Moog *et al.* 2002) and can have features such as entrance slit sizes that exclude most ants from entering the pre-formed domatia (Brouat *et al.* 2001). The physico-chemical characteristics of the cuticular waxes of *Macaranga* myrmecophytes (Müller & Riederer 2005, Dragota & Riederer

2007) also contribute to partner choice because only specific mutualistic ants can walk on the stems and branches that are covered by these waxes (Federle *et al.* 1997, 2002).

Research into ant–plant mutualisms has also discovered the phenomenon of host choice, which is exerted by the symbiont. Colony-founding ant queens (‘foundresses’) shed their wings as soon as they have decided on a specific nesting site (Hölldobler & Wilson 1990). Thus, foundresses of plant-ants cannot easily correct their decision for a certain host. Myrmecophytes (hosts) often are closely related to non-myrmecophytes (non-hosts) (see Blattner *et al.* 2001, Bänfer *et al.* 2004 for a phylogeny of myrmecophytic *Macaranga* species and Heil *et al.* 2004 for Mesoamerican acacias). Even myrmecophytes in the same genus, which in principle represent suitable hosts for a certain ant species, can strongly differ in their quality as a host (Heil *et al.* 2009), and nutrient availability and light conditions further define how much ant rewards an individual plant can produce (Heil *et al.* 2002, Bixenmann *et al.* 2011). Thus, foundresses of plant-ants must be able to distinguish hosts from non-hosts and good hosts from bad hosts. Because plant ants usually swarm at night and find their host over comparably large distances (Türke *et al.* 2010), many of them use volatile organic compounds (VOCs) in their host-finding behavior. The queens of both *Azteca* and *Allomerus* ants are attracted to the VOCs emitted by *C. nodosa* plants, which suggests that they use olfactory cues for the long range detection of their host plants (Edwards *et al.* 2006a). Similarly, *Crematogaster* foundresses are able to distinguish among the odors emitted from myrmecophytic and non-myrmecophytic *Macaranga* plants (Jürgens *et al.* 2006), and *Pheidole* queens use volatiles to distinguish their *Maieta* hosts from other, sympatric myrmecophytes (Dáttilo *et al.* 2009). *Pseudomyrmex triplarinus* ants use cuticular

compounds to distinguish their host tree *Triplaris americana* from non-host plant in the context of their pruning behavior (Weir *et al.* 2012). These signals could also be involved in host recognition for colony founding, although they are unlikely to serve as long-distance signals.

The fact that foundresses use plant odors to localize their hosts raises the question regarding the degree to which they can judge the quality of a plant as a future host. Recent olfactometer experiments demonstrated that foundresses of *P. ferrugineus* can use VOCs to distinguish their host *Acacia* plants from non-host species (Fig. 3). VOCs also allowed them to prefer a high-reward *Acacia* species over a low-reward species and healthy plants with high levels of reward production over damaged plants that produce lower amounts of EFN and FBs (Razo-Belmán & Heil 2012). In conclusion, queens of plant-ants clearly show host choice behavior that is based on VOCs, at least to some degree.

### HOST SANCTIONS? PLANTS SHED DOMATIA WHEN THEY ARE NOT BEING DEFENDED

Because obligate ant-plants nest in domatia which are localized in hollow shoots, thorns, or leaf pouches, the growth of the ant colony and thus its potential reproductive success depend strongly on the vegetative growth rate of their host plant (Fonseca 1993, 1994, Frederickson & Gordon 2009). Although this situation apparently enhances the temptation to cheat (see ‘castration mutualisms’), it also provides the plant with a possibility to punish inefficient exploiters. *Hirtella myrmecophila* produces leaf-pouches in the young parts of the branches and *Allomerus octoarticulatus* ants use these structures as domatia. *Hirtella* plants can use the abortion of domatia in older leaves as a strategy of host sanction mechanism (Izzo & Vasconcelos 2002). Similarly, simulated her-

bivory caused *C. nodosa* plants to shed stem domatia and thereby punishes non-defending inhabitants (Edwards *et al.* 2006b). However, it has been questioned (Weyl *et al.* 2010) whether these responses represent true sanction mechanism or should be better understood as effects of partner fidelity feedback (see below).

### COMPETITIVE SCREENING

Myrmecophytes in most genera can associate with more than one ant species and direct competition among ant colonies represents a crucial determinant of the colony that can dominate a certain host (Davidson & Snelling 1989, Palmer 2003, Palmer *et al.* 2003, Raine *et al.* 2004, Debout *et al.* 2005). In adaptation to this situation, some species of plant-ants have evolved cooperative colony founding (Izzo *et al.* 2009) or secondary polygyny (Feldhaar *et al.* 2000, 2005; Dalecky *et al.* 2005; Kautz *et al.* 2009b), the first adaptation facilitating colony establishment, the second one likely allowing an individual colony to occupy a given host longer than it would be allowed by the lifetime of a single queen.

Competition among foundresses represents an important determinant in the ant-plant mutualism, which opens the question whether myrmecophytes can employ competitive screening to associate preferably with good partners. An ideal test of screening would be to manipulate the cost of entry into the host and observe the subsequent evolutionary trajectories of mutualists and parasites (D. Yu, pers comm.). In practice, the mesoamerican ant-acacias with their positive assortment of high-reward hosts with high-quality mutualists (Heil *et al.* 2009) represent a natural experiment of this type. Indeed, observations over 7 mo demonstrated that mutualists are more likely than exploiters to finally dominate young plants of a high-reward species. Even within one species, the frequency at which mutualists could outcompete the exploiters was positively correlated to the initial rate of reward production by the individual plants (Heil 2013). Because more aggressive ants generally represent the better defenders (Xu & Chen 2010) and because the aggressiveness of most ant workers is limited by energy supply (see below), ant-plants can use the fact that the adapted mutualists are more efficient in making use of the specific food rewards to shift competitive balances in favor of the mutualists.

### SANCTIONS, RECIPROCAL REWARDING, OR PARTNER FIDELITY FEEDBACK? LESSONS FROM ANT-PLANT RESEARCH

Most myrmecophytes reduce the amounts of FBs or EFN produced when they are not well-defended by their resident ants. In some cases, the presence of ants *per se* can induce FB production via an as-yet unknown chemical mechanism (Risch & Rickson 1981, Heil *et al.* 1997). Similarly, mesoamerican acacias reduce EFN secretion when they are inhabited by the exploiter ant, *P. gracilis* (Heil *et al.* 2009, see Fig. 1). Alternatively, the harvesting of the FBs can directly enhance production rates (Folgarait *et al.* 1994), as it is also commonly being reported for nectar (Heil 2011): plants respond to an accumulation of EFN on the nectary

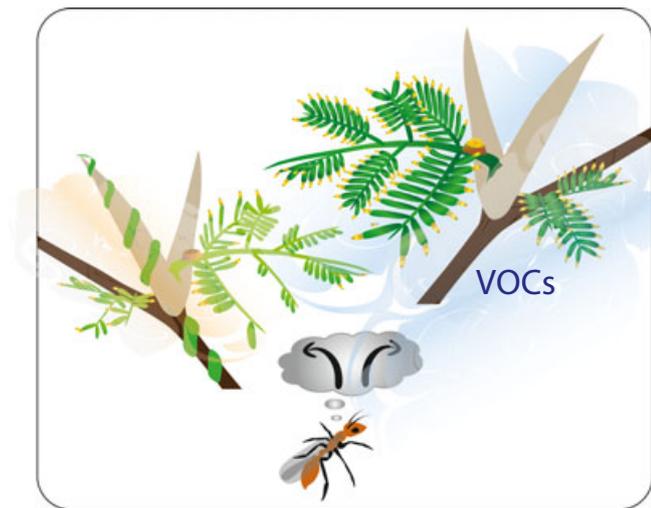


FIGURE 3. Host choice by foundresses of mutualistic ants: Colony-founding queens of the mutualistic ant, *Pseudomyrmex ferrugineus*, use volatile organic compounds (VOCs) that are emitted from their host to choose healthy individuals of a high-reward host species (Razo-Belmán & Heil 2012).

with a decrease in its *de novo* production (Heil *et al.* 2000, Bixenmann *et al.* 2011) or perhaps even with its re-absorption (Escalante-Pérez *et al.* 2012). The crucial involvement of invertase (catalyzing the hydrolysis of sucrose to yield glucose and fructose) in the secretion of both floral and EFN (Ruhlmann *et al.* 2010, Orona-Tamayo *et al.* 2013b) provides a possible mechanism for the re-absorption of monosaccharides that accumulate on the outside of a nectary.

The ants, however, are not passive consumers in these systems. Colony growth of the ants responds positively to FB supply (Itino *et al.* 2001), and plant-ants that are supplied with more EFN immediately enhance their aggressive behavior and, thus, their protective efficacy (González-Teuber *et al.* 2012, see Fig. 4). That is, both ant-plants and plant-ants exhibit phenotypic plasticity in the provisioning of rewards and services that amount to an efficient reciprocal rewarding system.

In most mutualisms, partner choice appears to be realized by the host. By contrast, studies on ant–plant mutualisms commonly describe the phenomenon of host choice, which is exerted by the symbiont (Fig. 3). Moreover, the efficiency of ant-mediated defence depends on the amount (and, likely, the quality) of rewards that ants receive (Fig. 4), a situation which can be described as partner sanctions. Consequently, research into ant–plant mutualisms demonstrates that actions and characteristics of the symbiont strongly contribute to the stable association of good hosts with high quality symbionts.

In the above sections, we summarized the different phenomena that make ant rewards less suitable for non-adapted exploiters, reduce the production of rewards when the legitimate consumer is absent (Fig. 1), or in which hosts seem to sanction cheating ants by actively shedding domatia. Unfortunately, the underlying physiological mechanisms remain to be discovered: we are not aware of any study that has found a compound, or trait, that is used by a myrmecophyte to identify its resident ant or to directly measure its actions, although plants in principle can perceive animal-derived compounds, such as egg-deposition glue (Hilker & Meiners 2006) or pheromones (Helms *et al.* 2013).

Independently of the detailed physiological mechanisms, however, it remains an open question whether the above-

described phenomena represent true host sanctions or specific adaptations that make the rewards exclusive, or whether they are consequences of partner fidelity feedback and ecological fitting. Partner fidelity feedback means that fitness-relevant traits of host and symbiont depend on each other and that this reciprocal dependency is enough to prevent cheating: each partner has a benefit from maintaining the other one in a good physiological state. By contrast, host sanctions apply when a (costly) punishment is required to prevent symbionts from cheating (Weyl *et al.* 2010). In fact, ant–plant mutualisms are likely to depend mainly on partner fidelity feedback. On the one hand, plants require healthy leaves to maintain the photosynthesis that allows for future growth, including the production of any type of tissue or organ, independently of whether or not they are myrmecophytes. Thus, undefended plants might simply lack the physiological capacities to invest in the production of new domatia or ant rewards. Moreover, plants commonly shed leaves as soon as these are too heavily damaged by herbivores or microorganisms. In fact, controlled cell death represents a paramount strategy in plant resistance to pathogens. On the other hand, ant workers are commonly limited by energy acquisition. Thus, increased EFN secretion rates have been related to higher survival rates of ant workers (Lach *et al.* 2009) and also increased the activity and aggressiveness of ants in facultative and in obligate ant–plant interactions (Sobrinho *et al.* 2002, Ness 2006, Ness *et al.* 2009, González-Teuber *et al.* 2012).

In summary, both the decrease in the production of ant rewards and domatia by undefended plants and the lower aggressiveness in ant colonies that receive less EFN are likely intrinsic physiological responses rather than specific adaptations to the mutualistic interaction. Even the biochemical ‘lock–key system’ that is formed by Kunitz-type PIs in the FBs of ant acacias and the chymotrypsin 1-like proteases in the ants, which are insensitive to these PIs, is not likely to represent the outcome of a co-evolutionary process (Orona-Tamayo *et al.* 2013a). Kunitz-type PIs are common in seeds of members of the Fabaceae and all of the few ant species that have been investigated so far possess chymotrypsin-like proteases, which are generally less sensitive to Kunitz-type PIs (Orona-Tamayo *et al.* 2013a). Thus, although sec-

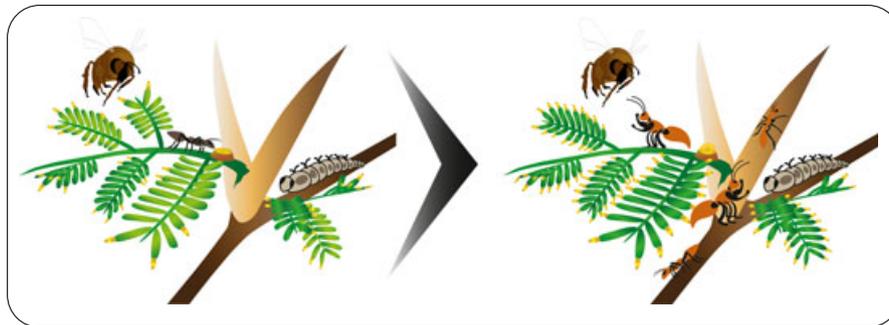


FIGURE 4. Partner sanctions by mutualistic ants: Adapted mutualist ants (*Pseudomyrmex ferrugineus*) respond to the level of reward provisioning by their *Acacia* host and defend their host more strongly against nectar robbers (such as bees) and herbivores (such as caterpillars) and – likely – exploiter ants when they receive more rewards, particularly extrafloral nectar (EFN) (Clement *et al.* 2008, Heil *et al.* 2009, González-Teuber *et al.* 2012).

ondary co-evolutionary adjustments can strengthen the reciprocal matching, ecological fitting as defined by Janzen (1985) is more likely to explain a large part of this phenomenon. Partner fidelity feedback (also termed ‘closed loop of fitness-relevant traits’, see Heil *et al.* 2009) turns out to represent an important stabilizing process, and its functioning does not necessarily require any specific adaptations. Mutualisms are generally formed among species from different kingdoms, each of which can produce something easily and at low costs that is required (and difficult to produce) by the other (Bronstein *et al.* 2006, Bronstein 2009, Leigh 2010). Think only of all the transportation mutualisms: plants are unlikely to evolve mobility, whereas animals are similarly unlikely to evolve photosynthesis as an efficient source of metabolically accessible energy; thus, the trade ‘energy for mobility’ serves all partners involved (Boucher *et al.* 1982, Bronstein 1994, Fleming & Holland 1998, Roopin *et al.* 2008) and each partner benefits from maintaining the other one in a good physiological state.

## PERSPECTIVES

Research into symbiotic ant–plant mutualisms has significantly contributed to our knowledge on factors that stabilize symbiotic mutualisms. Factors and mechanisms that have been described for ant–plant mutualisms include chemical characteristics and signalling mechanisms that facilitate the production of exclusive rewards, host choice, and partner sanctions (that is, control mechanisms exerted by the symbiont), and closed loops among fitness-relevant vital traits of host and symbiont that lead to stable partner fidelity feedback mechanisms. How likely are these mechanisms to apply also to other types of mutualisms and what should be done to further improve our knowledge on ant–plant mutualism?

One obvious benefit of studying ant–plant mutualisms lies in the fact that, although the mutualisms commonly are obligate in the long run, both partners can be separated to study each of them with and without the mutualistic interaction. Whereas it appears almost impossible to deprive a legume of its Rhizobia once nodules have been formed, plant-ants can be removed easily. Thus, both the formation and the dissolution of the mutualism can be experimentally simulated and its consequences for both partners studied independently. It might be no coincidence that many examples of partner sanctions and host choice stem from this research field. Furthermore, the rewards generally are easily accessible and thus available for direct analysis.

Tropical ant-plants provide researchers with benefits than are difficult to find in other mutualisms. Research into ant–plant mutualisms tells us that more caution is required before we assume specific strategies and adaptations to explain phenomena that contribute to the stability of mutualisms. A deeper mechanistic understanding and comparative research will be required to distinguish adapted (co-evolved) strategies from ecological fitting and partner fidelity feedback. Most obligate ant-plants are found in the same genera with non-myrmecophytic species and the same situation applies to plant-ants as well, allowing for direct comparative

approaches. Do non-myrmecophytic *Hirtella* or *Cordia* species shed heavily damaged and infected leaves as easily as the myrmecophytes? Do plant-ants show nutritional adaptations to their vegetarian lifestyle that cannot be observed in other arboreal ants? Are plant-ants biochemically and behaviorally adapted to defend their host, or are they simply expanding the use of compounds and behaviors used for their own protection to include their host as well? The possibility to separate both partners without destroying their physical integrity and the existence of symbiotic and non-symbiotic species in the same genera on the side of both ants and plants, make ant-plants highly suitable systems to study questions of general interest in the establishment and maintenance of mutualisms at the ecological and evolutionary level.

There are no reasons to assume, however, that mechanisms which stabilize an ant–plant mutualism are in principle different from those that stabilize other mutualisms. In fact, the recent discovery that pollinators can sanction against cheating flowers which produce less nectar but have unchanged morphological and olfactory traits (Brandenburg *et al.* 2012) demonstrates that partner sanctions function also in other types of mutualisms. Research into ant–plant mutualisms has proven highly successful in the identification and understanding of multiple mechanisms that are required to stabilize mutualisms in the presence of non-reciprocating exploiters.

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