Divergent investment strategies of *Acacia* myrmecophytes and the coexistence of mutualists and exploiters

Martin Heil[a,b,1], Marcia González-Teuber[b], Lars W. Clement[c], Stefanie Kautz[b], Manfred Verhaagh[d], and Juan Carlos Silva Bueno[a]

[a]Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Irapuato, Km. 9.6 Libramiento Norte, CP 36821, Irapuato, Guanajuato, México; [b]Department of General Botany-Plant Ecology, FB BioGeo, University of Duisburg-Essen, Universitätsstrasse 5, D-45117 Essen, Germany; [c]Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Hans-Knöll-Strasse 8, D-07745 Jena, Germany; and [d]Staatliches Museum für Naturkunde Karlsruhe, Erbprinzenstrasse 13, D-76133 Karlsruhe, Germany

Ant-plant interactions represent a diversity of strategies, from exploitative to mutualistic, and how these strategies evolve is poorly understood. Here, we link physiological, ecological, and phylogenetic approaches to study the evolution and coexistence of strategies in the *Acacia–Pseudomyrmex* system. Host plant species represented 2 different strategies. High-reward hosts produced significantly more extrafloral nectar (EFN), food bodies, and nesting space than low-reward hosts, even when being inhabited by the same species of ant mutualist. High-reward hosts were more effectively defended against herbivores and exploited to a lower extent by nondefending ants than low-reward hosts. At the phenotypic level, secretion of EFN and ant activity were positively correlated and a mutualistic ant species induced nectar secretion, whereas a nondefending exploiter did not. All of these mechanisms contribute to the stable association of high-reward hosts with defending ant species. However, exploiter ants are less dependent on the host-derived rewards and can colonize considerable proportions of the low-reward hosts. Mapping these strategies onto phylogenetic trees demonstrated that the low-reward hosts represent the derived clade within a monophyletic group of obligate ant plants and that the observed exploiter ant species evolved their strategy without having a mutualistic ancestor. We conclude that both types of host strategies coexist because of variable net outcomes of different investment–payoff regimes and that the effects of exploiters on the outcome of mutualisms can, thus, increase the diversity within the taxa involved.

ant plant | cheater | extrafloral nectar | indirect defense | parasite of mutualism

Mutualisms are interactions among different species that enhance the fitness of the involved partners and are often based on the exchange of rewards and services (1, 2). Other species can exploit such rewards without reciprocating, hence acting as "cheaters" or "parasites" of the mutualism (2–5). Such exploiters have been described, among others, for ant-plant mutualisms (6–9), cleaner fishes, lichens, pollination, mycorrhiza, and root–rhizobia symbiosis (see refs. 3, 4, and 10 for reviews). Exploiters do not invest in service provisioning and thus can be competitively superior to mutualists when receiving the same rewards (2, 3, 11–15). As a result, mutualists should tend to reduce service provisioning while optimizing the benefit obtained, leading to a strong "temptation to defect," and exploitation has been predicted to be common in high-reward mutualisms (4). However, exploiters pose problems for the stability of mutualisms when competing with true mutualists for suitable hosts.

Several mechanisms can stabilize mutualisms against exploitation. For example, host sanctions act at the level of phenotypic variation, allowing the host to adjust reward provisioning to the current quality of the service received. Such mechanisms stabi-

lize, for example, the mutualisms of host fishes with cleaner fishes (16) and plant roots with rhizobia (12, 17). Partner choice mechanisms, by contrast, depend on the recognition of species-specific cues during the phase of partner finding (18) and are known, among others, for ant-plant mutualisms (19, 20) and the early phases of signaling between plant roots and rhizobia (21, 22).

Exploiters evolving from former mutualists likely can retain the traits on which partner choice is based. Consequently, they are more efficiently controlled by host sanction mechanisms, which are based on the actual level of reciprocation. By contrast, exploiters with no history as a mutualist likely lack cues that specifically enable the onset of the interaction and can be excluded via partner choice (23). Because the phylogenetic history of an exploiter determines the mechanism that can stabilize a mutualism against its evolution and maintenance, we hereinafter use the term cheater explicitly for exploiters with mutualistic ancestors (24) and term those species parasites that invaded the system without having an evolutionary history as a mutualist (4, 23). Making this distinction it becomes apparent that few phylogenetic studies reported evidence for the existence of cheaters (3, 5), an obvious contradiction to theoretical expectations, which demonstrates the existence of mechanisms that can stabilize mutualisms.

We used a defensive ant-plant mutualism to investigate whether reward production by congeneric host plant species affects their level of exploitation. Such ant-plant mutualisms serve the "indirect defense" of plants against herbivores (25, 26) and allow studies of the ecology and evolution of mutualisms, because the partners involved can be experimentally separated (26). In detail we asked: (*i*) Do mutualists and exploiters compete for host plants? (*ii*) Do host species differ in their level of exploitation? (*iii*) Does reward production feed back to the level of exploitation and does the investment depend on the payoff received? (*iv*) Do the ants influence reward production? We used molecular phylogenies to understand the evolutionary history of the different strategies used by host plants and mutualistic and exploiting ants.

Results and Discussion

Competition for Host Plants. Mesoamerican *Acacia* myrmecophytes (obligate ant plants) provide nesting space in hollow
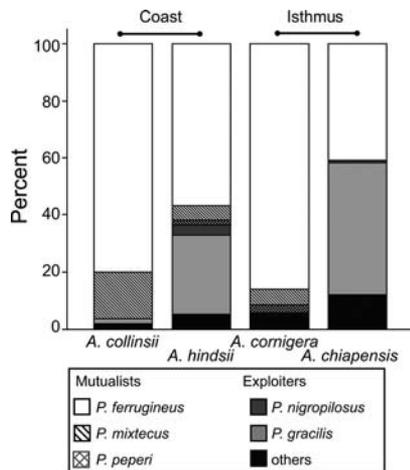
Fig. 1. Occupancy of *Acacia* hosts by different ant species. The relative proportion of plants inhabited by the different ant species is given separately for the 4 species of *Acacia* myrmecophytes that were investigated at the 2 sites, site 1 (Coast) and site 2 (Isthmus). Sample sizes are 52 for *A. collinsii*, 61 for *A. hindsii*, 90 for *A. cornigera*, and 115 for *A. chiapensis*.

thorns and food rewards [extrafloral nectar (EFN) and food bodies (FBs)] (27–29) to specific, defending *Pseudomyrmex* ants (6). Several closely related species are involved in the mutualism on the side of both ants and plants. Nondefending ants exploit this system and workers of the nondefending ant species, *Pseudomyrmex gracilis*, prevent mutualist queens from colony founding (6), which makes *P. gracilis* an active competitor for host plants. To document the competition among colony-founding queens, we deprived branches of *Acacia cornigera* and *Acacia hindsii* plants of their resident ants and quantified foundresses in the newly developed thorns. Both plant species and ant species had a significant effect on the number of thorns occupied by queens [general linear model (GLM): plant species: $F_{1,371} = 5.84$, $P = 0.016$; ant species: $F_{5,371} = 49.74$, $P < 0.001$; interaction: $F_{5,371} = 5.02$, $P < 0.001$]. After 6 weeks, a total of 205 foundresses had arrived on *A. cornigera* and 448 on *A. hindsii*, occupying on average 82% (*A. cornigera*) and 121% (*A. hindsii*) of the available thorns (121% for *A. hindsii* because numerous thorns were occupied by 2 queens). Among all queens observed, 3% of those found on *A. cornigera* and 5% of those on *A. hindsii* belonged to *P. gracilis*, whereas the majority was queens of the mutualists, *Pseudomyrmex ferrugineus*, *Pseudomyrmex mixtecus*, and *Pseudomyrmex peperi*. Because *Acacia* plants usually are dominated by a single ant colony (27) these data indicate a massive competition for host plants.

Next, we quantified the level of exploitation of 4 *Acacia* species: *A. hindsii* and *Acacia collinsii* were investigated at the Pacific coast, *A. cornigera* and *Acacia chiapensis* at a distance of ≈270 km in the Isthmus of Tehuantepec (both sites: state of Oaxaca, Mexico). In total, some 98% of the *A. cornigera* and *A. collinsii* plants were dominated by mutualistic *Pseudomyrmex* species. The most common ant was *P. ferrugineus*, which inhabited >80% of the plants of both species (Fig. 1). By contrast, *P. ferrugineus* inhabited only 55% of *A. hindsii* and 41% of *A. chiapensis*, whereas the parasite *P. gracilis* inhabited 26% and 44%, respectively, of plants of the latter 2 species (Fig. 1). Mutualists were significantly more frequent on *A. cornigera* and *A. collinsii* than on *A. hindsii* and *A. chiapensis*, both within each habitat and pooled over both habitats (likelihood ratio test, $G_{coast} = 14.1$, df = 1, $P < 0.0001$; $G_{isthmus} = 65.8$, df = 1, $P < 0.0001$; $G_{pooled} = 77.6$, df = 1, $P < 0.001$, mutualists and parasites pooled within classes).
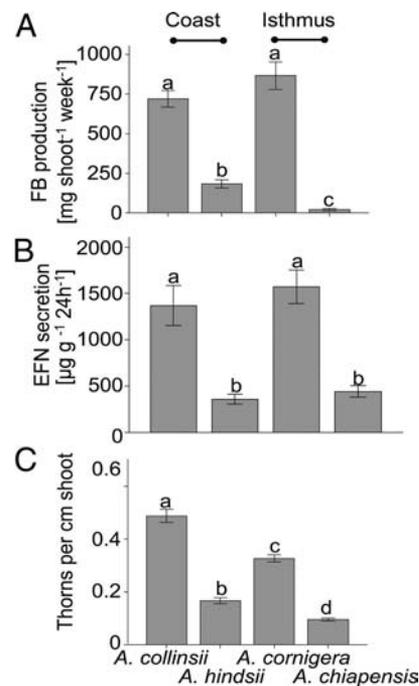

Fig. 2. Reward production by *Acacia* myrmecophytes. (*A*) FB production averaged for 8 plants per species is expressed in mg FB dry weight per shoot and week. (*B*) EFN secretion was averaged from 20–25 plants per species and is expressed in µg soluble solids per g leaf dry mass and day. (*C*) Nesting space was estimated for 30 plants per species as number of thorns present per cm shoot. Bars marked by different letters are significantly different ($P < 0.05$ according to LSD posthoc test after univariate ANOVA). Bars indicate means; error bars indicate standard errors.

Plant-Derived Ant Rewards. Reward production by the 4 myrmecophytes was quantified at the same 2 sites. To control for effects of the ant inhabitant, only plants inhabited by *P. ferrugineus* were used. Plant species was a significant source of variation in the FB production rates (GLM: $F_{3, 21} = 101.57$, $P < 0.001$). *A. collinsii* and *A. cornigera* produced on average 720 and 870 mg of FBs per shoot and week, whereas *A. hindsii* produced 180 mg of shoot^{-1}·wk^{-1} and *A. chiapensis* produced only 25 mg of shoot^{-1}·wk^{-1}. The production rates of *A. hindsii* and *A. chiapensis* were significantly [$P < 0.05$, least significant difference (LSD) posthoc tests] lower than those of *A. collinsii* and *A. cornigera* (Fig. 2*A*). The same pattern was found for EFN secretion, because *A. cornigera* and *A. collinsii* secreted ≈3 times more EFN (1,400 and 1,600 µg of soluble solids g^{-1} leaf dry mass·24 h^{-1}) than *A. hindsii* and *A. chiapensis* (<500 µg of g^{-1}·24 h^{-1}) (GLM: $F_{3,92} = 19.176$, $P < 0.001$; Fig. 2*B*). Similar to food provision, *A. cornigera* and *A. collinsii* had more thorns per cm shoot than *A. hindsii* and *A. chiapensis* (GLM: $F_{3,120} = 142.05$, $P < 0.001$; Fig. 2*C*).

In short, *A. cornigera* and *A. collinsii* can be characterized as high-reward hosts, whereas *A. hindsii* and *A. chiapensis* are low-reward hosts. All plants investigated here were inhabited by the same ant species, and 1 high-reward species and 1 low-reward species co-occurred in each of the sites. Neither site effects nor the ant inhabitant can therefore explain these differences, which obviously represent species-specific traits.

Reward Provisioning, Defense, and Exploitation. To investigate whether plants gain an enhanced payoff from higher investment rates we chose 23 *A. cornigera* and 24 *A. hindsii* plants, all being inhabited by *P. ferrugineus*. EFN secretion was quantified on the main shoot, and plant stems were then damaged to simulate attack. Numbers of ants recruiting to these lesion sites were
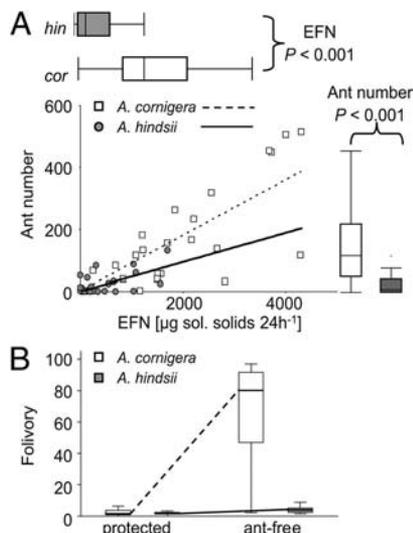
Fig. 3. EFN secretion, ant activity, and defense. (*A*) Numbers of *P. ferrugineus* workers passing near fresh lesions are depicted as a function of EFN secretion of the respective plant separately for *A. cornigera* and *A. hindsii*. The distribution of data are indicated by box-whisker plots and differed significantly between the plant species (Mann–Whitney-*U* test: $P < 0.001$). (*B*) The higher activity of ants on the high-reward species *A. cornigera* translates to a higher defensive effect, because leaf damage (calculated as percentage of damaged leaflets) suffered by this species increased significantly more over 3 weeks of ant exclusion than for the low-reward species *A. hindsii*.

quantified as a measure of colony aggressiveness during the next 2 h. Data on EFN secretion confirmed earlier results (Fig. 2*A*), because EFN secretion by *A. cornigera* was significantly higher than that by *A. hindsii* (Mann–Whitney $U = 58.0, P < 0.001$). For both species, numbers of ants recruited were positively correlated with EFN secretion (Fig. 3*A*; *A. hindsii*: Pearson's correlation coefficient $= 0.574$, $n = 24$, $P = 0.003$; *A. cornigera*: Pearson's correlation coefficient $= 0.748, n = 23, P < 0.001$), and significantly more ants were thus recruited on *A. cornigera* than on *A. hindsii* (Mann–Whitney $U = 71.5, P < 0.001$).

Different slopes of the regression lines describing the number of ants recruited as a function of EFN production (Fig. 3*A*) pointed to different defensive efficacies of EFN secretion for the 2 host species. This was tested by calculating the quotient of ant number per EFN secretion for each individual plant. These quotients for *A. cornigera* were significantly higher than for *A. hindsii* (Mann–Whitney $U = 141.0, P = 0.018$). Thus, a given increase in EFN provisioning increases ant recruitment to lesions more strongly on *A. cornigera* than on *A. hindsii*.

Resident ants being active on the plant surface should protect their host from competing ants (30) and *A. cornigera* was less often exploited than *A. hindsii* (Fig. 1), but does this ant activity translate to a stronger defense against herbivores? *P. ferrugineus* ants were excluded from 1 branch each of 10 *A. hindsii* and 10 *A. cornigera* plants. The resulting increase in folivory was indeed higher for *A. cornigera* than for *A. hindsii* (GLM on arcsin-transformed data: ant presence: $F_{1,59} = 70.862, P < 0.001$; plant species: $F_{1,59} = 55.635, P < 0.001$; interaction: $F_{1,59} = 54.207, P < 0.001$; see Fig. 3*B*), indicating that ants protect the high-reward host *A. cornigera* more strongly from folivory than *A. hindsii*.

Phylogenetic Relationships. We used sequence data to reconstruct the relationships among mutualistic, exploiting, and generalist ant species and among low-reward and high-reward hosts. Two exploiting ant species, *P. gracilis* and *Pseudomyrmex nigropilosus*, belong to the same genus as the mutualists but formed a clade well separated from the mutualists (Fig. 4*A*). These species likely
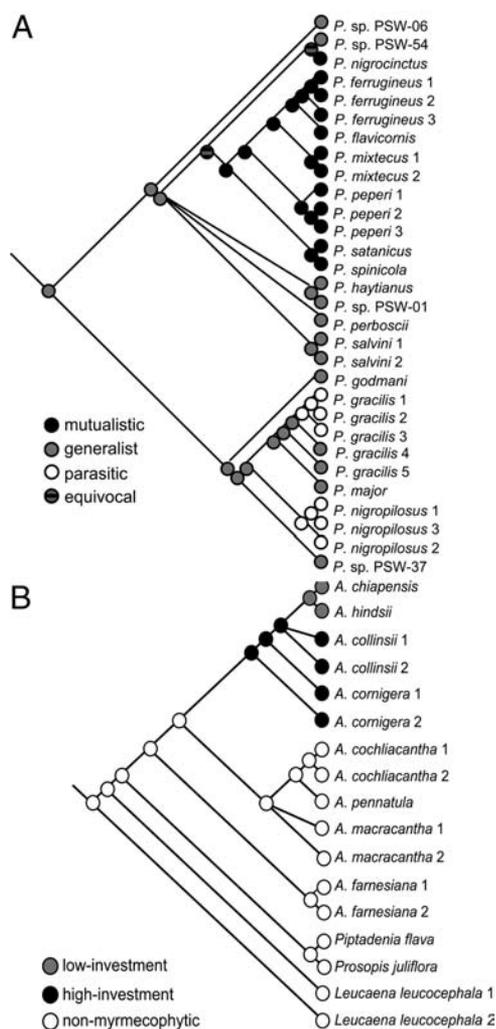

Fig. 4. Phylogenetic histories. (*A*) Associations of ants with *Acacia* myrmecophytes (mutualistic, generalist, and parasitic) traced on a phylogeny of *Pseudomyrmex* ants, as inferred from a 5-gene fragments analysis (*wg*, *abd*-A, LW *Rh*, 28S, and mtCOI, totaling 3,224 bp). Redrawn from ref. 23. (*B*) The investment strategies of myrmecophytic acacias and their relation to non-myrmecophytic species were traced on a phylogeny based on 2 gene fragments (*trnK* intron and *trnL-trnF* intrageneric spacer totaling 3,222 bp). Data were reevaluated from ref. 29. See Ref. 23 and Fig. S2 for branch support.

have no evolutionary history as a mutualist and represent parasites of the mutualism, which remains true also for a third exploiter of this system, *Camponotus planatus* (8). Thus, no evidence for a cheater exists so far, demonstrating the stability of the Mesoamerican *Acacia–Pseudomyrmex* mutualism against the evolution and persistence of exploiters that are derived from mutualists (but see refs. 31–33 for African ant acacias).

Character tracing for the hosts revealed that the low-reward species, *A. hindsii* and *A. chiapensis*, form a later diverging clade within the monophyletic group of obligate myrmecophytes (Fig. 4*B*) and led to the conclusion that the low-reward hosts exhibit the evolutionarily derived strategy. In fact, symbiotic mutualisms can show a tendency to relaxation or even be reversed toward a free-living lifestyle under certain external selection pressures (5).

The phylogeny of the ants used here is based on mitochondrial and nuclear genes and was consistent with morphological findings (23). By contrast, the current molecular phylogeny of Mesoamerican *Acacia* myrmecophytes is based on chloroplast genes only and thus represents exclusively the maternal line, not allowing the detection of hybridization processes. An alternative
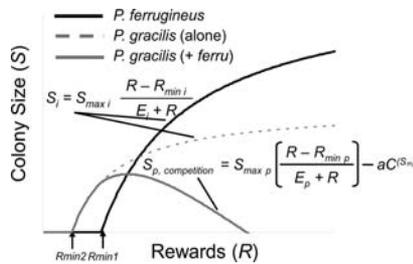
Heil et al.

Fig. 5. Colony size as a function of plant reward level. Relations among reward level *R*, ant colony size *S* of species *i*, and the efficiency E_i at which rewards are converted into workers were inferred from models based on Michaelis–Menten functions (*SI Text*) with the intrinsic maximum colony size being $S_{max i}$, and $R_{min i}$ describing the minimum reward level at which a colony can exist. When both species compete for a high-reward host, the positive feedback of rewards to protecting behavior (Fig. S3) allows the mutualist ($i = m$) to displace the parasite ($i = p$), as indicated here by a term that exponentially increases with the mutualists' colony size, where *a* and *C* are constants. Black line indicates *P. ferrugineus*; dotted gray line indicates *P. gracilis*; gray line indicates *P. gracilis* in the presence of a competitively superior *P. ferrugineus* colony.

hypothesis would be that introgression of nonmyrmecophytic *Acacia* species into high-reward species gave rise to the species with intermediate levels of ant rewards, which we term low-reward hosts. Amplified fragment-length polymorphism (AFLP) data revealed high genetic similarity between the low-reward *A. hindsii* and the high-reward *A. collinsii* (29), and both species share elongate inflorescences as a typical morphological trait (27). Although sequence data are regarded more suitable to reconstruct phylogenetic trees than AFLP data, the current phylogenetic information does not allow the exclusion of alternative topologies to the one presented here (Fig. 4*B*). Most importantly in the context of the present study, high-reward and low-reward hosts formed part of the same major phylogenetic clade, and species using the different strategies were found to coexist at the same sites.

Stabilizing the *Acacia*–*Pseudomyrmex* Mutualism. High-reward hosts were better protected from herbivory and exploitation than low-reward hosts and no cheaters could be detected. These observations empirically support that, for the evolution and stabilization of mutualisms, investment must depend on the payoff received (2) and that phenotypically plastic partners must exchange reciprocal responses (34).

Differences in the degree of reciprocal adaptations among the partners appear a key factor that tightens the association of high-reward hosts with defending ants. Mutualist *Pseudomyrmex* ants are physiologically adapted to live exclusively on host-derived food rewards (6, 20, 23) and were never found nesting outside an *Acacia* host (27, 35). By contrast, the common exploiter, *P. gracilis*, can nest independently of *Acacia* hosts (35) and consumes food that is captured off the host plant (6). We used Michaelis–Menten functions to describe how ant colony size increases as a function of reward level and the efficiency at which rewards are converted into workers. These functions demonstrate that the exploiter can reach competitive superiority when host-derived rewards limit colony growth, whereas the mutualist appears superior at higher reward levels (Fig. 5 and *SI Text*). Interestingly, models expressing the reproduction rate of a mutualist population in terms of costs and net effects as a function of its partner's population size revealed similar predictions when net effects to one partner were assumed to increase asymptotically and saturate with increasing abundance of the other partner (36).

Net Effects of Exploitation and Species Coexistence. If high-reward hosts are much more likely inhabited by defending mutualists, why do low-reward hosts exist at all, and does this facilitate the observed coexistence of both strategies? External selection pressures might force mutualisms to evolve toward independent lifestyles (5) and an expensive defense pays off best when there is a high herbivore pressure. The costs of ant rewards are illustrated by their rapid reduction in the absence of herbivores (33), their dependency on nutrient supply (37), and numerous mechanisms that regulate their production (38–42). EFN secretion was positively correlated with worker activity of the mutualist *P. ferrugineus* (Fig. S1*A*; *A. hindsii*: Pearson's correlation coefficient = 0.733, *n* = 41, *P* < 0.001; *A. cornigera*: Pearson's correlation coefficient = 0.879, *n* = 22, *P* < 0.001), whereas there was a negative, yet insignificant, relation with the presence of *P. gracilis* workers on *A. hindsii* (Fig. S1*B*; Pearson's correlation coefficient = −0.193, *n* = 41, *P* = 0.226). Both the correlation coefficient and the slope of the regression line were higher for *A. cornigera* than for *A. hindsii* (Fig. S1*A*), pointing to a stronger inducing effect of *P. ferrugineus* ants on EFN secretion by the first host species. An ant-exclusion experiment confirmed that *Acacia* myrmecophytes quickly reduce EFN secretion when mutualists are missing and this effect differs among plant species: *P. ferrugineus* workers were excluded from single branches of *A. cornigera* and *A. hindsii* and these branches secreted significantly less EFN than branches to which ants had access (Fig. S1*C*). Plant species and presence of ants had a significant effect on EFN secretion (GLM: plant species: $F_{1,21} = 40.015$, $P < 0.001$; ant presence: $F_{1,21} = 24.884$, $P < 0.001$; interaction: $F_{1,21} = 7.072$, $P = 0.016$) and the effect of mutualist ants on EFN secretion differed between the plant species (as indicated by the significant interaction), increasing EFN secretion more on *A. cornigera* than on *A. hindsii*.

In short, the presence of mutualists positively affects EFN secretion, although at different degrees among host species, whereas the exploiter lacks the reward-inducing features. Most likely, the plants somehow monitor the presence and/or defensive activity of the mutualist ants. In Mesoamerican *Acacia* myrmecophytes, leaf damage does not increase EFN secretion because resting levels of the wound hormone, jasmonic acid, are sufficient to elicit full secretion rates (29). Likewise, a regulation via EFN removal appears unlikely, because the parasite also feeds on EFN. The cue remains to be identified that is used by the mutualist ants to modulate EFN secretion or by the hosts to monitor the presence of reciprocating ants. However, it is clear that, as predicted before (34), phenotypic plasticity plays a key role in stabilizing the high-reward mutualisms in our study system. This interpretation is in line with a model showing that herbivore pressure poses selective pressure on the nesting space provided by myrmecophytic *Tachigali* species (43). In general, adaptive responses by mutualists to variation in partner quality can maintain diversity within mutualist guilds (31), and costs rather than benefits appear to be the central factor that shapes mutualisms (44). Spatial and temporal variation in herbivore pressure and the costs of defense apparently allow the coexistence of alternative strategies (31, 32).

Conclusions. Exploiters might gain a competitive advantage from not reciprocating when reward provisioning by the host is not adjusted to the nature of the interacting partner. In the *Acacia*–*Pseudomyrmex* system, by contrast, rewards are preferentially provided to mutualist ants. Positive feedback mechanisms (Fig. S3) among phenotypically plastic and fitness-relevant traits of both partners facilitate associations of high-reward host plants with defending ant species and stabilize the *Acacia*–*Pseudomyrmex* interaction against the evolution of cheaters and the exploitation by parasites (Fig. 5).

Plants using the high-reward strategy appeared better protected against exploitation (see Fig. 1 and *SI Text*) and should be superior under conditions that make exploitation by nondefending ants particularly costly. By contrast, defense investment can become relaxed under conditions making defense function poorly (45). Because costs and benefits of defense depend on numerous and variable environmental factors such as nutrient availability and herbivore pressure, high-reward hosts and low-reward hosts can coexist at the same sites, at least in the current ecological situation (Figs. 1 and 2).

We found no hints that exploitation was more common in high-reward mutualisms and no phylogenetic evidence for the existence of cheaters. Thus, our study lends empirical support to the prediction that mutualisms are stabilized when increased investments in a partner increase reciprocation. Mutualisms can be stabilized by the reciprocal exchange of phenotypically plastic responses among partners.

Materials and Methods

Study System. The study was conducted at 2 field sites in Oaxaca, Mexico. All plants were 1.0–2.0 m high. Site 1 (Pacific coast) was ≈15 km W of Puerto Escondido in the coastal area of the state (≈15°55 N and 097°09 W, elevation 15 m), and site 2 (Isthmus) was in the Isthmus of Tehuantepec ≈25 km N of Matias Romero (≈17°06 N and 094°55 W, elevation 150 m). *A. collinsii* Safford, *A. hindsii* Bentham, and *A. cornigera* (L.) Willdenow grow at site 1, and *A. hindsii*, *A. cornigera* and *A. chiapensis* Safford grow at site 2. The mutualist ant species *P. ferrugineus* F. Smith, *P. mixtecus* Ward, and *P. peperi* Forel cannot be found nesting apart from their host plants. Other ant species (at the study sites mainly *P. gracilis* Fabricius and *P. nigropilosus* Emery) inhabit the hollow thorns and consume FBs, EFN, and insect prey but show no defending behavior, hence acting as exploiters (6, 7).

Competition for Host Plants. We investigated colonization by excluding ants from branches of 18 *A. cornigera* and 39 *A. hindsii* plants on site 1 in October 2007. Inhabited thorns were cut off and resident ants were excluded from the shoot tips by applying a ring of Tangletrap (Tanglefoot). After 6 weeks, newly produced thorns were counted and censused for founding queens.

Ant Occupancy. A total of 316 plants at both sites were censused during April and May 2004 for ant inhabitants (n = 112 plants at site 1 and 204 plants at site 2). A colony was scored as inhabiting a plant only when thorns contained living brood and when no workers of other ant species were observed on the plant.

Reward Production. Reward production was investigated at site 1 for *A. collinsii* and *A. hindsii* and at site 2 for *A. cornigera* and *A. chiapensis*. FB production was quantified for 8 individuals per species in September 2005. Ants were excluded from the main shoots, which were then placed into gauze bags. After 3 weeks, all newly produced leaves were collected and FBs were counted. A subsample of 100 FBs per plant was dried and weighed to determine average FB dry weight to calculate FB production in dry mass $shoot^{-1} \cdot week^{-1}$. EFN secretion was quantified in September 2005 on 20–25 plants per species as described (29, 40). Thorn numbers were counted and overall shoot length was measured to the nearest 5 cm for 30 plants per species to calculate the number of thorns per cm shoot.

EFN Secretion and Ant Activity. Ant activity in the undisturbed stage was quantified on 18 *A. cornigera* and 39 *A. hindsii* plants inhabited by *P. ferrugineus* at site 1 in September 2006 as follows. Six lines were drawn in the upper, central, and lower third of the main shoot of each plant and ants passing these lines were counted 3 times for 1 min each. Thereafter, EFN secretion was quantified for the same plants. To investigate the effect of mutualist ants on EFN secretion, *P. ferrugineus* ants were excluded from 1 branch of each of 5 *A. cornigera* and 6 *A. hindsii* plants (site 1, April 2008) and EFN secretion was quantified on the ant exclusion and the nearest untreated branch after 2 weeks.

The relation of EFN secretion with numbers of ants being recruited to lesions was studied at the same site in March 2007. EFN secretion of the main shoots of 24 *A. hindsii* and 23 *A. cornigera* plants was quantified and plants then were mechanically damaged (cutting a line ≈2 cm long and 3 mm deep) in the upper, central, and lower region of the main stem. To quantify the level of aggressiveness at which the plant is defended by its resident colony, workers passing lines ≈1 cm above and below each lesion site were censused 3 times for 1 min each with ≈1 h between censuses. Plant protection from herbivores was studied on site 1 in April 2008. Ants were excluded from 1 branch of each of 10 *A. hindsii* and 10 *A. cornigera* plants. Three leaves were collected from each of these and the neighboring ant-inhabited branches after 3 weeks, and the numbers of intact and damaged leaflets were counted to calculate folivory as percentage of damaged leaflets.

Reconstruction of Ancestral States. We inferred the phylogeny of hosts by using a Bayesian approach and a maximum-likelihood analysis based on *trnL-trnF* intron and *trnK* intron sequence data (29). Nucleotide substitution models were selected by using Akaike Information Criterion (46). Branch support was assessed as Bayesian posterior probabilities and bootstrap support resulting from the maximum-likelihood analysis. Reconstruction of ancestral states (47) of the high-reward and the low-reward strategy was conducted as described (23). See *SI Text* for detailed information on phylogenetic methods. See ref. 23 and Table S1 for GenBank accession numbers, and see ref. 23 and Fig. S2 for branch support.

1. Bronstein JL (1994) Our current understanding of mutualism. *Q Rev Biol* 69:31–51.
2. Doebeli M, Knowlton N (1998) The evolution of interspecific mutualisms. *Proc Natl Acad Sci USA* 95:8676–8680.
3. Sachs JL, Simms EL (2006) Pathways to mutualism breakdown. *Trends Ecol Evol* 21:585–592.
4. Bronstein JL (2001) The exploitation of mutualisms. *Ecol Lett* 4:277–287.
5. Douglas AE (2008) Conflict, cheats and the persistence of symbioses. *New Phytol* 177:849–858.
6. Clement LW, Köppen S, Brand WA, Heil M (2008) Strategies of a parasite of the ant-*Acacia* mutualism. *Behav Ecol Sociobiol* 26:953–962.
7. Janzen DH (1975) *Pseudomyrmex nigropilosa*: A parasite of a mutualism. *Science* 188:936–937.
8. 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.
9. Tillberg CV (2004) Friend or foe? A behavioral and stable isotopic investigation of an ant-plant symbiosis. *Oecologia* 140:506–515.
10. Yu DW (2001) Parasites of mutualisms. *Biol J Linn Soc* 72:529–546.
11. Doebeli M, Hauert C, Killingback T (2004) The evolutionary origin of cooperators and defectors. *Science* 306:859–862.
12. Kiers ET, Rousseau RA, West SA, Denison RF (2003) Host sanctions and the legume–rhizobium mutualism. *Nature* 425:78–81.
13. Hoeksema JD, Bruna EM (2000) Pursuing the big questions about interspecific mutualism: A review of theoretical approaches. *Oecologia* 125:321–330.
14. Sachs JL, Mueller UG, Wilcox TP, Bull JJ (2004) The evolution of cooperation. *Q Rev Biol* 79:135–160.
15. Doebeli M, Hauert C (2005) Models of cooperation based on the Prisoner's Dilemma and the Snowdrift game. *Ecol Lett* 8:748–766.
16. Bshary R, Grutter AS (2005) Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biol Lett* 1:396–399.
17. Simms EL, et al. (2006) An empirical test of partner choice mechanisms in a wild legume–rhizobium interaction. *Proc R Soc London Ser B* 273:77–81.
18. Foster KR, Wenseleers T (2006) A general model for the evolution of mutualisms. *J Evol Biol* 19:1283–1293.
19. 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 London Ser B* 268:2131–2141.
20. Heil M, Rattke J, Boland W (2005) Postsecretory hydrolysis of nectar sucrose and specialization in ant/plant mutualism. *Science* 308:560–563.
21. Mithöfer A (2002) Suppression of plant defense in rhizobia–legume symbiosis. *Trends Plants Sci* 10:440–444.
22. Limpens E, Bisseling T (2003) Signaling in symbiosis. *Curr Opin Plant Biol* 6:343–350.
23. Kautz S, Lumbsch HT, Ward PS, Heil M (2009) How to prevent cheating: A digestive specialization ties mutualistic plant–ants to their ant-plant partners. *Evolution* 63:839–853.
24. Segraves K, Althoff D, Pellmyr O (2005) Limiting cheaters in mutualism: Evidence from hybridization between mutualist and cheater yucca moths. *Proc R Soc London Ser B* 272:2195–2201.
25. Chamberlain SA, Holland JN (2009) Quantitative synthesis of context dependency in ant-plant protection mutualisms. *Ecology* 90:2384–2392.
26. Heil M, McKey D (2003) Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annu Rev Ecol Evol Syst* 34:425–453.

27. Janzen DH (1974) *Swollen-Thorn Acacias of Central America* (Smithsonian Institution Press, Washington, DC).
28. Heil M, Baumann B, Krüger R, Linsenmair KE (2004) Main nutrient compounds in food bodies of Mexican *Acacia* ant plants. *Chemoecology* 14:45–52.
29. Heil M, et al. (2004) Evolutionary change from induced to constitutive expression of an indirect plant resistance. *Nature* 430:205–208.
30. Palmer TM (2004) Wars of attrition: Colony size determines competitive outcomes in a guild of African acacia ants. *Anim Behav* 68:993–1004.
31. Palmer TM, Stanton ML, Young TP (2003) Competition and coexistence: Exploring mechanisms that restrict and maintain diversity within mutualist guilds. *Am Nat* 162:S63–S79.
32. Palmer TM (2003) Spatial habitat heterogeneity influences competition and coexistence in an African acacia ant guild. *Ecology* 84:2843–2855.
33. Palmer TM, et al. (2008) Breakdown of an ant-plant mutualism follows the loss of large herbivores from an African savanna. *Science* 319:192–195.
34. Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. *Science* 294:321–326.
35. Ward PS (1993) Systematic studies on *Pseudomyrmex* acacia ants (Hymenoptera: Formicidae: Pseudomyrmecinae). *J Hym Res* 2:117–168.
36. Holland JN, DeAngelis DL, Bronstein JL (2002) Population dynamics and mutualism: Functional responses of benefits and costs. *Am Nat* 159:231–244.
37. Heil M, et al. (2002) Nutrient allocation of *Macaranga triloba* ant plants to growth, photosynthesis, and indirect defense. *Funct Ecol* 16:475–483.
38. Heil M, et al. (1997) Food body production in *Macaranga triloba* (Euphorbiaceae): A plant investment in antiherbivore defense via mutualistic ant partners. *J Ecol* 85:847–861.
39. Risch SJ, Rickson F (1981) Mutualism in which ants must be present before plants produce food bodies. *Nature* 291:149–150.
40. 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.
41. Heil M, et al. (2001) 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.
42. Mondor EB, Addicott JF (2003) Conspicuous extra-floral nectaries are inducible in *Vicia faba. Ecol Lett* 6:495–497.
43. Fonseca CR (1993) Nesting space limits colony size of the plant ant *Pseudomyrmex concolor. Oikos* 67:473–482.
44. Bronstein JL (2001) The costs of mutualism. *Am Zool* 41:825–839.
45. Agrawal AA, Fishbein M (2008) Phylogenetic escalation and decline of plant defense strategies. *Proc Natl Acad Sci USA* 105:10057–10060.
46. Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: Advantages of akaike information criterion and Bayesian approaches over likelihood ratio tests. *Syst Biol* 53:793–808.
47. Pagel M (1999) The maximum-likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Syst Biol* 48:612–622.