Supporting Information

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SI Text

Positive Feedback Mechanisms Stabilize an Obligate Mutualism. Our data provide empirical evidence for the prediction that making the investments by a mutualist dependent on the payoff received can stabilize mutualisms against exploitation. In our study system, the 2 high-reward host species (Fig. 2) were less frequently exploited by nondefending ants (Fig. 1) than the 2 low-reward hosts, and the high-reward *A. cornigera* gained relatively more protection from its increased investments than did the low-reward *A. hindsii* (Fig. 3*B*). Apparently, investment pays off, although at degrees differing among host species, but why are parasitic ant species less successful on high-reward hosts? EFN secretion was positively correlated with the worker activity of the mutualist *P. ferrugineus*. We found experimental support for a modulating effect of mutualist ants on EFN secretion by host plants (Fig. S1). However, this induction effect depended on the host species. By contrast, the parasitic *P. gracilis* apparently lacks the EFN-inducing features (Fig. S1). Similar phenomena have been reported for the FB production of *Piper*, *Macaranga*, and *Cecropia* myrmecophytes (1–3) and EFN secretion of *M. tanarius* (4).

An induction of EFN secretion by ants cannot, however, explain the differences among host species that we report here (Fig. 2), because only plants inhabited by *P. ferrugineus* were used for that part of the study. Competition among mutualists and exploiters together with different degrees of specialization is, therefore, most likely stabilizing the association of high-reward hosts with mutualists and the coexistence of both strategies (5, 6).

Studies on various ant-plant mutualisms have demonstrated that (*i*) ant protection increases the sizes of the host plants and, thus, food reward levels (7, 8), (*ii*) increased host plant size and food rewards increase ant colony size (7, 9, 10), and (*iii*) increasing numbers of defending ants improve the protection from herbivores (10–12) and competitors (13). Although ant fitness and plant fitness are not directly linked to each other, fitness-relevant parameters of both partners in the *Acacia*–*Pseudomyrmex* system and other ant-plant mutualisms form, thus, a closed loop of positive feedback mechanisms (Fig. S3). In our study system, these interactions are tightened by evolutionary changes that make EFN secretion by Mesoamerican *Acacia* myrmecophytes independent of induction by herbivory (8), the very direct correlation of the amount of EFN secreted with the level of ant activity (Fig. 3*A*), and the capacity of mutualistic, but not parasitic, ants to induce EFN secretion (Fig. S1).

Differences in the degree of reciprocal adaptations appear to be the key factor leading to the tight association of the high-reward host species with the defending ant species: Mutualist *Pseudomyrmex* have never been found nesting outside a myrmecophytic *Acacia* plant (refs. 14 and 15 and personal observations by P.S. Ward and M.H.) and depend entirely on the plant-derived food (16). By contrast, the most common parasite in our study system, *P. gracilis*, can nest independently of *Acacia* hosts (15) and regularly consumes food that is captured off the host plant (16). *P. gracilis* produces invertase and other digestive enzymes that *P. ferrugineus* lacks (17, 18) and therefore can use external food sources, but presumably is less efficient in doing so because of the need of coping with more different types of food.

Mutualists thus should be superior competitors on high-reward hosts. We illustrate this idea by using a Michaelis–Menten function to describe how colony size S of species i increases as a function of plant reward level R, the efficiency E_i at which rewards are converted into workers, the intrinsic maximum colony size $S_{max\ i}$, and the minimum reward level $R_{min\ i}$ at which the colony can exist (Fig. 5).

$$S_i = S_{max\ i}\frac{R - R_{min\ i}}{E_i + R}.$$

Because the parasite can also use external food sources and is not a plant-reward specialist, we expect $R_{min\ m} > R_{min\ p}$ and $E_m > E_p$ (subscript m standing for mutualist and p for parasite). Also, *P. gracilis* shifts to reproduction earlier and has smaller colonies (16), so $S_{max\ m} > S_{max\ p}$. When both species compete for a high-reward host plant, the strong positive feedback of rewards to protecting behavior allows the mutualist to displace the parasite, as indicated here by an exponentially increasing term that grows with the mutualist ant's colony size, where a and C are constants.

$$S_{p,\ competition} = S_{max\ p}\left(\frac{R - R_{min\ i}}{E_p + R}\right) - aC^{(Sm)}.$$

In contrast, on low-reward host plants, the parasitic ant's ability to use external food sources can make it competitively superior and eventually allows the parasite to dominate. In summary, we expect mutualists to dominate on high-reward hosts whereas parasites can dominate a considerable percentage of the low-resource hosts, an association pattern that indeed has been observed in the field (Fig. 1).

Phylogenetic Inference. For the phylogenetic analyses of *Acacia* species we used both a Bayesian approach and a maximum-likelihood analysis. The Bayesian (B/MCMC) analyses were performed by using MrBayes 3.1.2 (19). Posterior probabilities were approximated by sampling the trees using a Markov chain Monte Carlo (MCMC) method. The sequences were tested for the most appropriate model of nucleotide substitution analyses by MrModeltest version 2.3 (www.abc.se/~nylander/) using Akaike Information Criterion. The most appropriate maximum-likelihood models of evolution were GTR+ Γ (*trnL*–*trnF* intron), and GTR+ I (*trnK* intron). The dataset was partitioned and each partition was allowed to have its own model parameters as proposed by Nylander et al. (20). No molecular clock was assumed. A run with 4,000,000 generations starting with a random tree and using 12 simultaneous chains was executed. Every 100th tree was saved into a file. The first 300,000 generations (i.e., the first 3,000 trees) were deleted as the “burn in” of the chain. We plotted the log-likelihood scores of sample points against generation time by using TRACER 1.0 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure that stationarity was reached after the first 300,000 generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium value (21). Of the remaining 74,000 trees (37,000 from each of the parallel runs) a majority rule consensus tree with average branch length was calculated by using the “sumt” option of MrBayes. Posterior probabilities were obtained for each clade. The maximum-likelihood analyses were performed with GARLI version 0.951 (http://www.nescent.org/informatics/download.php?software_id=4). For the combined dataset, the model GTR+I was determined to fit the data best because running partitions is not possible with GARLI. Bootstrap support was based on 2,000 replications. We considered only those clades as well supported that have a posterior probability of at least 0.95 and bootstrap support ≥70%. Congruence between the datasets was assessed by comparing bootstrap support of clades >70% for each locus (22). The combined alignment is available in TreeBASE (www.treebase.org/treebase). Sequences were available from an earlier study (8). GenBank accession numbers are given in Table S1. Phylogenetic trees were drawn by using TREEVIEW (21).

Reconstruction of Ancestral States. High-reward, low-reward, and nonmyrmecophytic ancestral states of the taxa included were reconstructed based on a 2-gene fragment phylogeny (*trnL-trnF* intron and *trnK* intron, with a total of 3,222 bp). Ancestral states were reconstructed with maximum likelihood as the optimality criterion on 1,000 trees sampled with B/MCMC (as described above) by using the Trace Character Over Trees option in Mesquite 2.01 (http://mesquiteproject.org). Using a likelihood ratio test, the asymmetric 2-parameter model was selected for this analysis. Only ancestral states reconstructed with raw likelihood scores >2.0 (i.e., the default setting T = 2.0 in Mesquite), corresponding to a conservative approximation of proportional likelihood values >0.95 in our analysis, were considered to be significant following Edwards (23).

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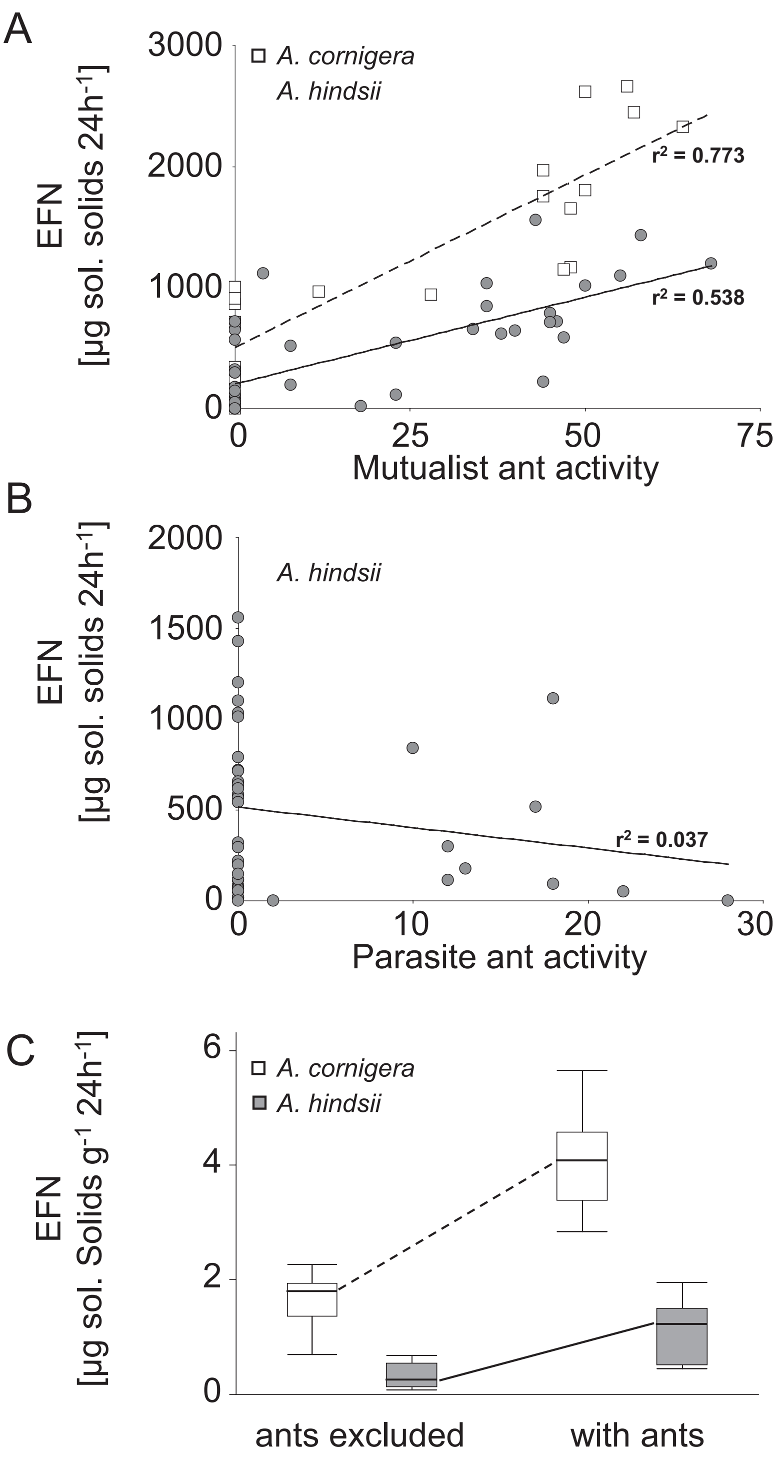

Fig. S1. Induction of EFN secretion by mutualist *P. ferrugineus* ants. (*A*) EFN secretion of the main shoot expressed in μg soluble solids per 24 h is depicted as a function of the mutualist ant activity separately for the high-reward host *A. cornigera* and the low-reward host *A. hindsii*. The higher slope and the better fit of the regression line for *A. cornigera* indicate a stronger dependency among EFN secretion and the activity and number of mutualist ants in this species than in the case of *A. hindsii*. (*B*) By contrast, the presence of the parasite, *P. gracilis*, had a slightly negative, although insignificant, effect on EFN secretion. The parasite obviously lacks the EFN-inducing features. (*C*) The different response of EFN secretion by *A. cornigera* and *A. hindsii* to the presence of mutualists (*A*) is confirmed by an ant-exclusion experiment, in which *A. cornigera* branches with and without ants differed much more strongly in EFN secretion (μg soluble solids per g leaf dry mass and 24 h) than did branches of *A. hindsii*.

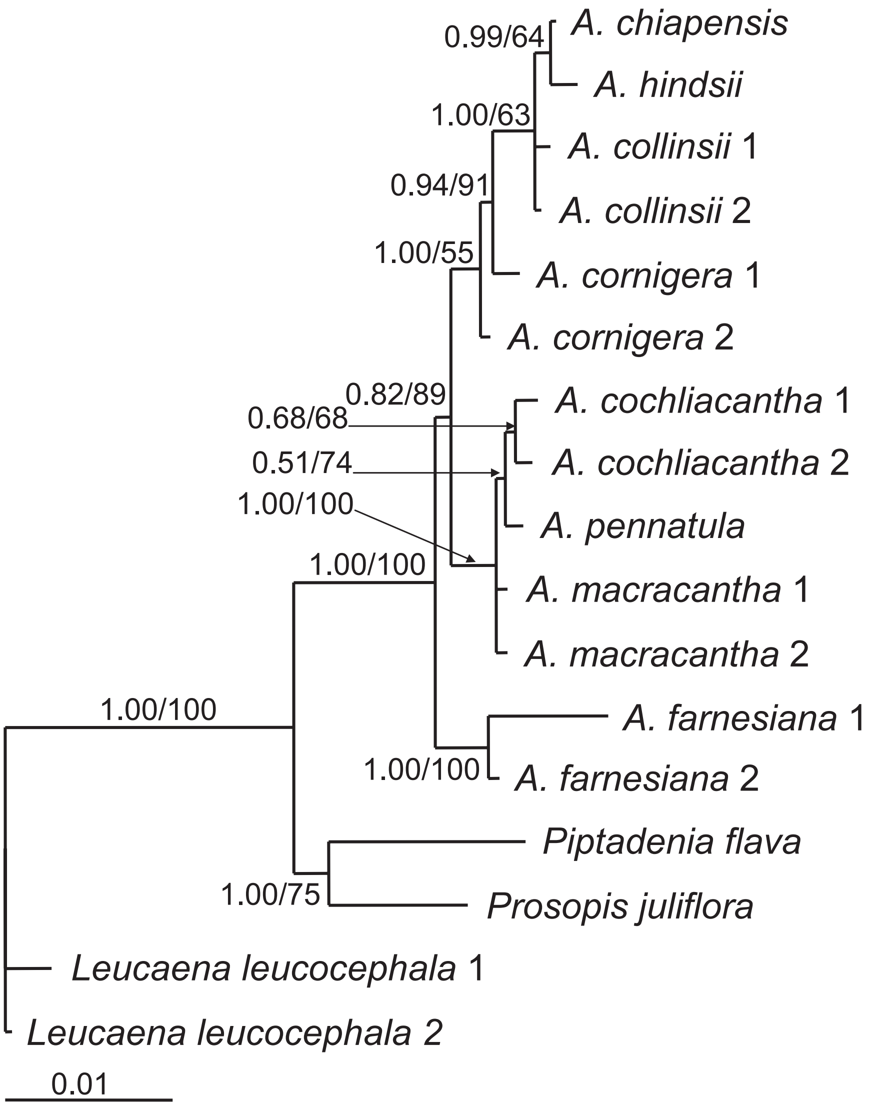

Fig. S2. Phylogeny of *Acacia* species as inferred from a 2-gene partition analysis (3,222 bp). This is a 50% majority rule consensus tree based on 74,000 trees from a B/MCMC tree sampling procedure. Numbers at branches indicate posterior probabilities/likelihood bootstrap support.

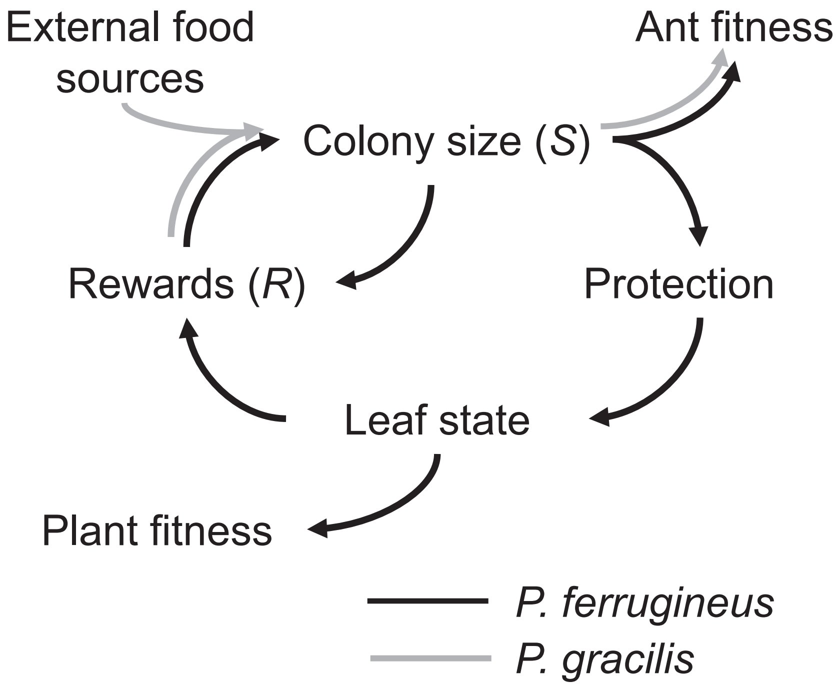

Fig. S3. Positive feedback among fitness-relevant traits. Ant colony size of the mutualist (black arrows) and plant leaf state are connected by a closed loop of positive feedback mechanisms, because colony size (S) determines the level of protection, which in turn determines plant leaf state and ant reward production. Ants thus have a positive effect on plant reward level R, whereas the rewards quantitatively feed back to ant colony size. The relations for the parasite (gray arrows) do not form a closed loop, because *P. gracilis* uses external food, does not protect the plant, and is not able to induce EFN secretion. Increased colony sizes of *P. gracilis*, thus, do not increase reward production levels.

Table S1. Species included in the phylogenetic inference of *Acacia* species and related nonmyrmecophytes

Species	Investment	GenBank accession no.	
		trnL-*trnF* intron	*trnK* intron
Acacia chiapensis	Low reward	AY574114	AY574097
Acacia cochliacantha 1	Nonmyrmecophyte	AY574112	AF274133
Acacia cochliacantha 2	Nonmyrmecophyte	AY574109	AY574094
Acacia collinsii 1	High reward	AY574107	AY574092
Acacia collinsii 2	High reward	AY574108	AY574093
Acacia cornigera 1	High reward	AY574121	AY574105
Acacia cornigera 2	High reward	AY574120	AY574104
Acacia farnesiana 1	Nonmyrmecophyte	AF195688	AF523115
Acacia farnesiana 2	Nonmyrmecophyte	AY574119	AY574103
Acacia hindsii	Low reward	AY574116	AY574099
Acacia macracantha 1	Nonmyrmecophyte	AY574117	AY574100
Acacia macracantha 1	Nonmyrmecophyte	AY574118	AY574101
Acacia pennatula	Nonmyrmecophyte	AY574111	AY574096
Leucaena leucocephala 1	Nonmyrmecophyte	AF278493	AF523094
Leucaena leucocephala 2	Nonmyrmecophyte	AY574106	AY574102
Piptadenia flava	Nonmyrmecophyte	AY574110	AY574095
Prosopis juliflora	Nonmyrmecophyte	AY574115	AY574098

Indicated are the species and specimens, their investment strategy, and the type of ant-plant mutualism and the GenBank accession numbers for the 2 gene fragments used for the study.