

Supplemental Data

S1

Interaction Intimacy Affects Structure and Coevolutionary Dynamics in Mutualistic Networks

Paulo R. Guimarães, Jr., Victor Rico-Gray,
Paulo S. Oliveira, Thiago J. Izzo,
Sérgio F. dos Reis, and John N. Thompson

Supplemental Experimental Procedures

Ant-Plant Interactions: Detailed Description

We used as a reference system the interactions between ants and plants (Figure S1). Ants are a dominant part of animal biodiversity in almost all terrestrial environments, and it is therefore not surprising that ant-plant interactions are geographically widespread and common in many ecological communities [S1]. Ants have been shown to be important in plant defense against herbivores, are relevant seed dispersers in deserts and forests, and promote selective gardening of plants (reviewed in [S1, S2]). In general, through their

sheer diversity, ants have evolved a wide range of mutualistic interactions with plants and have evolved vary broadly in their degree of interaction intimacy [S2]. Some ant-plant mutualisms are symbiotic, such as those between myrmecophytes and their protective ants [S3]. In such cases, interacting individuals show long-term physical contact with partners during their lifespan, and the ant-plant interaction involves specialized structures within plant hosts to house the symbionts and specialized physiologies in symbiotic ants to cope with the nutritional problems of completing development on a single host individual [S2, S4]. In contrast, other interactions are nonsymbiotic, such as those between ants and fleshy fruits, in which

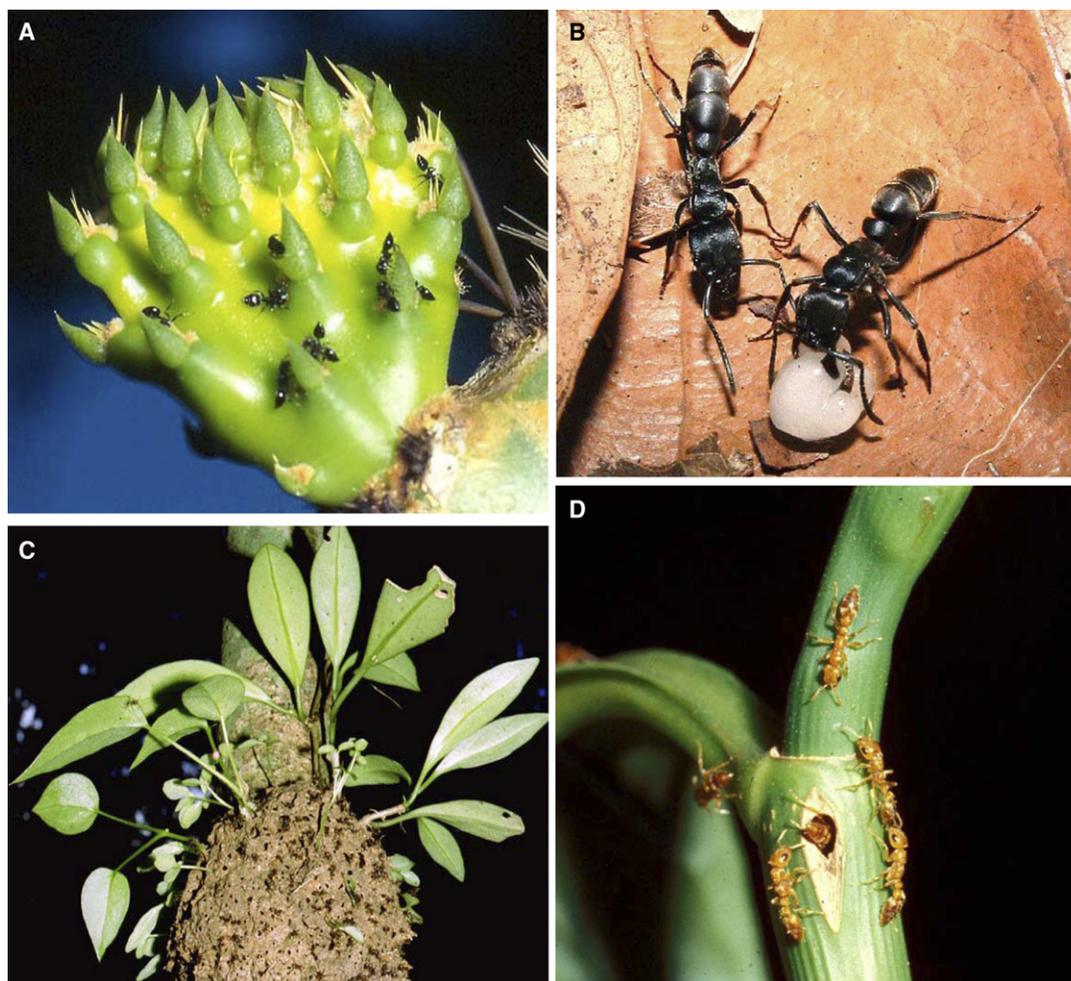


Figure S1. Examples of the Diversity of Ant-Plant Interactions

(A and B) Nonsymbiotic interactions between ants and plants. (A) shows *Crematogaster brevispinosa* ants visiting extrafloral nectaries of *Opuntia stricta* (Cactaceae) in a Mexican sand dune. (B) shows *Pachycondyla striata* ants removing primarily vertebrate-dispersed fruits of *Urera baccifera* (Urticaceae) on the floor of a Brazilian forest.

(C and D) Symbiotic interactions. (C) shows an ant-built carton nest within an epiphyte's spherical root mass in the Amazon forest, Brazil; seeds harvested by the ants are incorporated into the nest walls and germinate to form an ant-garden. (D) shows *Pseudomyrmex triplarinus* ants inhabiting myrmecophytic *Triplaris americana* (Polygonaceae) in a Brazilian forest. Photo credits are as follows: P. S. Oliveira (A and D), M. Gonzaga (B), and T. Guerra (C).

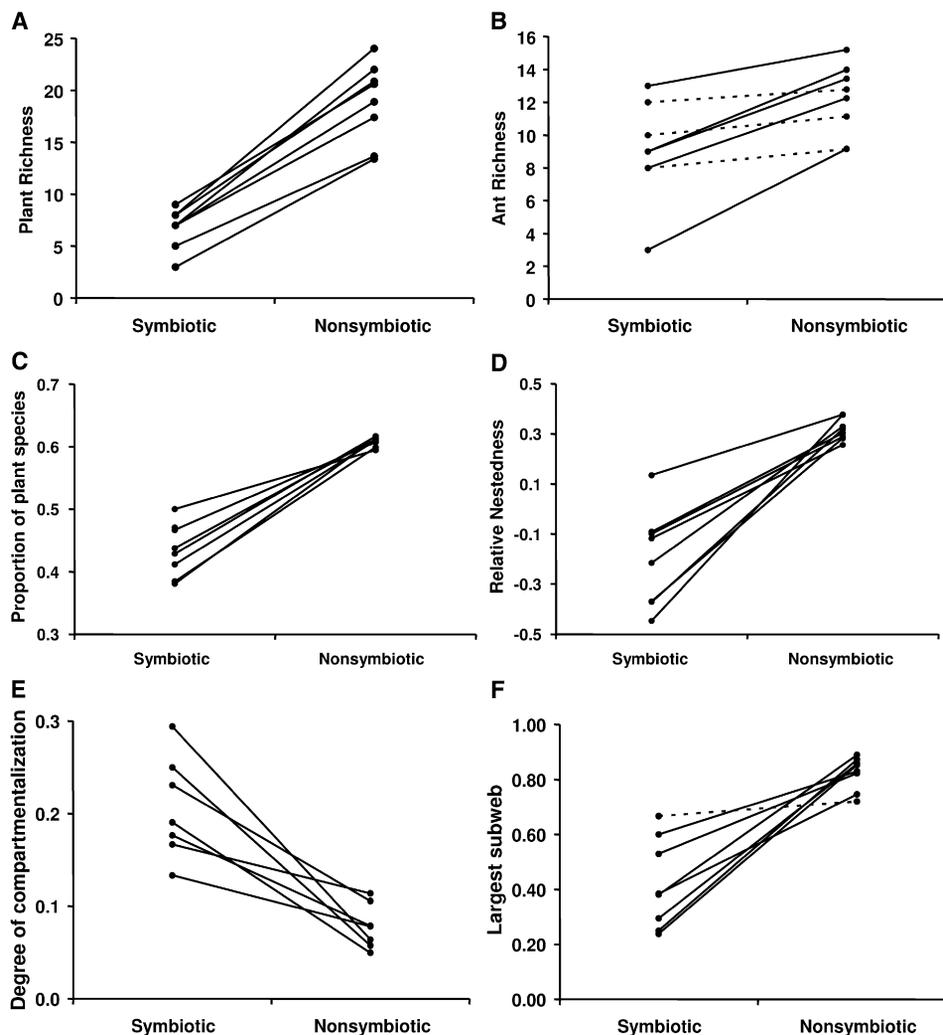


Figure S2. Comparisons between a Nonsymbiotic Network after Rarefaction of Sampling Effort and Eight Symbiotic Networks

(A–F) Each line corresponds to the difference between the value recorded for a symbiotic network and the mean value recorded for 100 rarefied networks derived from the nonsymbiotic network. Continuous line indicates that the probability of a rarefied network reproducing the value observed for a symbiotic network is lower than 5%, whereas a dashed line indicates that this probability is higher than 5%. (A) shows plant species richness.

(B) Ant species richness.

(C) Species-richness ratio, indicating the proportion of plant species in the network.

(D) Relative nestedness.

(E) The degree of compartmentalization, G , in which $G = (W - 1) / S$, in which W is the number of isolated subwebs and S is the total species.

(F) Size of largest isolated subweb. Note that all comparisons between symbiotic and rarefied nonsymbiotic networks show a similar trend for a given metric, e.g., all symbiotic networks show lower relative nestedness than their rarefied nonsymbiotic counterparts ($p < 0.01$ for all metrics, binomial test).

individuals of plant and ant species might establish short-term interactions with several to many partners of different species [S5, S6].

A recent study demonstrated that interactions between ants and myrmecophytes lead to networks with higher ecological specialization than those observed in interactions between ants and extrafloral nectar-producing plants [S7]. These results support the notion that symbiotic interactions (ant-myrmecophyte) are more specialized than nonsymbiotic interactions (ant-extrafloral nectar-producing plants) [S8]. We have extended that analysis by (1) performing comparative analyses of the structure of ant-plant mutualistic networks, searching for patterns of covariation between interaction intimacy and some basic structural aspects of the networks, and (2) investigating through numerical simulations the implications of the structure observed for coevolutionary processes.

Our dataset includes 19 networks that describe the patterns of specialization among species in mutualistic interactions between

ants and plants within communities (Table S1). Our symbiotic networks include the interactions between ants and myrmecophytes ($n = 11$), tank bromeliads ($n = 1$), and ant-garden plants ($n = 1$). Our nonsymbiotic networks include interactions between ants and fruits ($n = 2$) and extrafloral bearing plants ($n = 4$) (Table S1). Because sampling biases are a key factor affecting the results and conclusions of the study of mutualistic networks [S9, S10], we performed two set of structural analyses. First, we searched for differences in network structure between symbiotic and nonsymbiotic networks for the complete dataset. Second, to partially control for sampling biases, we first compared symbiotic interactions between ants and myrmecophytes and the nonsymbiotic interactions between ants and extrafloral nectar-producing plants sampled by us (T.J.I. and V.R.-G., unpublished data). Then, we performed rarefaction analyses to control the effects of sampling effort [S11].

Table S1. Ant-Plants Networks Investigated in This Study

Network	Ants Interacting with	Type	Locality	Reference
1	Myrmecophytes	Symbiotic	Amazon forest, Brazil	(T.J.I., unpublished data)
2	Myrmecophytes	Symbiotic	Amazon forest, Brazil	(T.J.I., unpublished data)
3	Myrmecophytes	Symbiotic	Amazon forest, Brazil	(T.J.I., unpublished data)
4	Myrmecophytes	Symbiotic	Amazon forest, Brazil	(T.J.I., unpublished data)
5	Myrmecophytes	Symbiotic	Amazon forest, Brazil	(T.J.I., unpublished data)
6	Myrmecophytes	Symbiotic	Amazon forest, Brazil	(T.J.I., unpublished data)
7	Myrmecophytes	Symbiotic	Amazon forest, Brazil	(T.J.I., unpublished data)
8	Myrmecophytes	Symbiotic	Amazon forest, Brazil	(T.J.I., unpublished data)
9	Myrmecophytes	Symbiotic	Amazon forest, Brazil	[S3]
10	Myrmecophytes	Symbiotic	Amazon forest, Peru	[S19]
11	Myrmecophytes	Symbiotic	Tropical forest, Costa Rica	[S20]
12	Tank bromeliads	Symbiotic	Amazon forest, Venezuela	[S21]
13	Ant garden's plants	Symbiotic	Rainforest, Malaysia	[S22]
14	Fleshy fruits	Nonsymbiotic	Atlantic forest, Brazil	[S6]
15	Fleshy fruits	Nonsymbiotic	Atlantic forest, Brazil	[S5]
16	EFN ^a -bearing plants	Nonsymbiotic	Tropical dry forest, Mexico	[S23]
17	EFN ^a -bearing plants	Nonsymbiotic	Sand dune matorral, Mexico	[S24]
18	EFN ^a -bearing plants	Nonsymbiotic	Semiarid environment, Mexico	[S14]
19	EFN ^a -bearing plants	Nonsymbiotic	Montane forest, Mexico	[S25]

^a EFN, extrafloral nectary.

Network Representation

Plant-animal interactions can be represented as two-mode networks, in which there are two sets of entities, i.e., plant and animal species. An ant-plant network is defined by an adjacency matrix R describing interactions between A ant species and P plant species in a well-defined ecological assemblage, in which $r_{ij} = 1$ if the ant i interacts with the plant species j and $r_{ij} = 0$ otherwise [S12, S13]. Therefore, our analysis of network structure focuses only on structural aspects that can be characterized by the presence or absence of an interaction in the network [S13]. We recognize that the intensity of interactions is another fundamental aspect for understanding the dynamics of interspecific networks [S7]. Nevertheless, the qualitative structure of the network allows us to describe basic and previously nondescribed differences among symbiotic and nonsymbiotic networks and allows a conservative and robust estimation of network structure and dynamics.

Controlling for Sampling Biases

(1) Sampling Biases due to Basic Aspects of Natural History

Sampling procedures might vary among interactions because of basic aspects of their natural history. For example, interactions between ants and fleshy fruits might occur in any area of the forest, and therefore, we usually sample these interactions by searching for events of interactions in the forest floor [S6]. In contrast, mutualisms in which ants defend plants always occur within the plants, and therefore, sampling in these defensive mutualisms is often based on the identification of potential interacting plants and then their inspection for recording ants [S14]. These different sampling methods might affect the structural patterns observed in mutualistic networks. We controlled for potential sampling biases by repeating all

analyses, but now contrasting only defensive mutualisms described by two of us (eight ant-myrmecophyte networks sampled by T.J.Z. and four ant-extrafloral nectar-producing plants interactions sampled by V.R.-G.).

Symbiotic, ant-myrmecophyte networks (hereafter myrmecophyte networks) showed lower total and plant species richness than nonsymbiotic interactions between ants and extrafloral nectar-producing plants (hereafter EFN networks), but no significant difference was recorded for ant species richness (Table S2). Accordingly, myrmecophyte networks showed lower (negative) relative nestedness and higher number of isolated subwebs than EFN networks (Table S2). Finally, the largest isolated subweb in a myrmecophyte network included, on average, less than 50% of ant and plant species, contrasting with the very large subwebs observed in EFN networks, which included, on average, almost 90% of all species in the network (Table S2).

(2) Sampling Biases Due to Sampling Effort

Differences in sampling effort might markedly affect the structure of mutualistic networks [S9, S11]. We performed rarefaction analyses to investigate the effects of sampling effort on comparisons between structural properties of symbiotic and nonsymbiotic networks [S11]. To carry out rarefaction analyses, we needed information about the number of interaction events for each ant-plant interaction (i.e., number of records of a given ant-fruit interaction), thereby reducing our dataset to one nonsymbiotic and eight symbiotic networks. We rarefied a network describing the nonsymbiotic interactions between ants and fleshy fruits in the Atlantic Forest, Brazil ([S6] and M.A.P., unpublished data) and randomly reducing the number of interaction events recorded in this nonsymbiotic network (817 interaction events) down to the number of events recorded in each

Table S2. Mean, Lower and Upper Values for Structural Metrics in Defensive, Ant-Plant Mutualisms

	Symbiotic (n = 8)	Nonsymbiotic (n = 4)	p
Ant species richness	9 (3–13)	14 (5–28)	0.174
Plant species richness	6.75 (3–9)	48.75 (12–99)	0.002
Total species richness	15.75 (6–21)	62.75 (17–127)	0.010
Significant nestedness (% of networks)	0%	75%	0.018
Relative nestedness	−0.20 (−0.45–0.13)	0.16 (−0.23–0.34)	0.020
Number of isolated subnetworks	4.25 (2–6)	2.25 (1–4)	0.037
Proportion of species richness in the largest isolated subnetwork	0.42 (0.24–0.67)	0.82 (0.29–1.00)	0.015

Symbiotic mutualisms are represented by Amazonian interactions between ants and myrmecophytes, and nonsymbiotic mutualisms are represented by interactions between ants and extrafloral nectar-producing plants in different Mexican ecological communities. All p values were obtained through two-independent samples randomization tests (n = 10,000 randomizations).

symbiotic myrmecophyte network sampled by T.J.Z. (44.4 ± 15.2 interaction events, $n = 8$ networks). We generated 100 rarefied nonsymbiotic networks for each symbiotic network.

Rarefaction analyses indicate that all structural differences between symbiotic and nonsymbiotic networks previously reported are not explained by differences in sampling effort. Symbiotic myrmecophyte networks show lower plant species richness, ant richness, and proportion of plants among interacting species than their rarefied nonsymbiotic counterparts (Figures S2A–S2C). Species richness might affect both nestedness [S12] and the number of subwebs (the maximum number of subwebs is limited by species richness). To control for the effects of species richness on structural comparisons between symbiotic and nonsymbiotic networks, we used relative nestedness (see Experimental Procedures in the main text for further details) and a standardized degree of compartmentalization (G), $G = (W - 1)/S$, in which W is the number of isolated subwebs and S is the total species richness. Hence, G varies from zero if the network is formed by a single subweb to close to one if the network is fragmented in many isolated subwebs. Myrmecophyte networks showed lower relative nestedness and a higher degree of compartmentalization than rarefied networks (Figures S2D and S2E). In fact, the number of subwebs was higher for symbiotic networks than for rarefied networks even without controlling for species-richness effects ($p = 0.04$, binomial test). In addition, the size of largest isolated subweb was higher in rarefied networks than in myrmecophyte networks (Figure S2F).

We emphasize that the analyses do not allow us to control for several distinct sampling biases that might affect the recorded network structure; such biases include those derived from spatiotemporal aggregation (see [S9, S15, S16] for a further discussion). However, our analyses provided additional support for our preliminary description of the relevance of interaction intimacy for the structure of mutualistic network. In addition, ant-plant networks do not show typical sampling biases, such as taxonomic and ecological lumping, observed in other ecological networks [S17, S18].

Supplemental References

- S1. Rico-Gray, V., and Oliveira, P.S. (2007). *The Ecology and Evolution of Ant-Plant Interactions* (Chicago: University of Chicago Press).
- S2. Bronstein, J.L., Alarcon, R., and Geber, M. (2006). The evolution of plant-insect mutualisms. *New Phytol.* **172**, 412–428.
- S3. Fonseca, C.R., and Ganade, G. (1996). Asymmetries, compartments and null interactions in an Amazonian ant-plant community. *J. Anim. Ecol.* **65**, 339–347.
- S4. Heil, M., and Mckey, D. (2003). Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology and Systematics* **34**, 425–553.
- S5. Passos, L., and Oliveira, P.S. (2003). Interactions between ants, fruits and seeds in a restinga forest in south-eastern Brazil. *J. Trop. Ecol.* **19**, 261–270.
- S6. Pizo, M.A., and Oliveira, P.S. (2000). The use of fruits and seeds by ants in the Atlantic forest of southeast Brazil. *Biotropica* **32**, 851–861.
- S7. Blüthgen, N., Menzel, F., Hovestadt, T., Fiala, B., and Blüthgen, N. (2007). Specialization, constraints, and conflicting interests in mutualistic networks. *Curr. Biol.* **17**, 341–346.
- S8. Thompson, J.N. (2005). *The Geographic Mosaic of Coevolution* (Chicago: The University of Chicago Press).
- S9. Olesen, J.M., and Jordano, P. (2002). Geographic patterns in plant-pollinator mutualistic networks. *Ecology* **83**, 2416–2424.
- S10. Ollerton, J., and Cranmer, L. (2002). Latitudinal trends in plant-pollinator interactions: are tropical plants more specialised? *Oikos* **98**, 340–350.
- S11. Blüthgen, N., Menzel, F., and Blüthgen, N. (2006). Measuring specialization in species interaction networks. *BMC Ecol.* **6**, 9.
- S12. Guimarães, P.R., Rico-Gray, V., dos Reis, S.F., and Thompson, J.N. (2006). Asymmetries in specialization in ant-plant mutualistic networks. *Proc. R. Soc. Lond. B. Biol. Sci.* **273**, 2041–2047.
- S13. Bascompte, J., Jordano, P., Melian, C.J., and Olesen, J.M. (2003). The nested assembly of plant-animal mutualistic networks. *Proc. Natl. Acad. Sci. USA* **100**, 9383–9387.
- S14. Rico-Gray, V., Palacios-Rios, M., Garcia-Franco, J.G., and Mackay, W.P. (1998). Richness and seasonal variation of ant-plant associations mediated by plant-derived food resources in the semiarid Zapotitlan Valley, Mexico. *Am. Midl. Nat.* **140**, 21–26.
- S15. Vazquez, D.P., and Aizen, M.A. (2004). Asymmetric specialization: A pervasive feature of plant-pollinator interactions. *Ecology* **85**, 1251–1257.
- S16. Vazquez, D.P., and Aizen, M.A. (2003). Null model analyses of specialization in plant-pollinator interactions. *Ecology* **84**, 2493–2501.
- S17. Martinez, N.D. (1993). Effects of resolution on food web structure. *Oikos* **66**, 403–412.
- S18. Abarca-Arenas, L.G., and Ulanowicz, R.E. (2002). The effects of taxonomic aggregation on network analysis. *Ecol. Modell.* **149**, 285–296.
- S19. Davidson, D.W., Snelling, R.R., and Longino, J. (1989). Competition among ants for myrmecophytes and the significance of plant trichomes. *Biotropica* **21**, 64–73.
- S20. Davidson, D.W., and Fisher, B.L. (1991). Symbiosis of ants with *Cecropia* as a function of light regime. In *Ant-Plant Interactions*, C.R. Huxley and D.F. Cutler, eds. (Oxford: Oxford University Press).
- S21. Blüthgen, N., Verhaagh, M., Goitia, W., and Blüthgen, N. (2000). Ant nests in tank bromeliads - an example of non-specific interaction. *Insectes Sociaux* **47**, 313–316.
- S22. Kaufmann, E., Weissflog, A., Hashim, R., and Maschwitz, U. (2001). Ant-gardens on the giant bamboo *Gigantochloa scortechinii* (Poaceae) in West-Malaysia. *Insectes Sociaux* **48**, 125–133.
- S23. Rico-Gray, V. (1993). Use of plant-derived food resources by ants in the dry tropical lowlands of coastal Veracruz, Mexico. *Biotropica* **25**, 301–315.
- S24. Diaz-Castelazo, C., Rico-Gray, V., Oliveira, P.S., and Cuautle, M. (2004). Extrafloral nectary-mediated ant-plant interactions in the coastal vegetation of Veracruz, Mexico: Richness, occurrence, seasonality and ant foraging patterns. *Ecoscience* **11**, 472–481.
- S25. Diaz-Castelazo, C., and Rico-Gray, V. (1998). Frecuencia y estacionalidad en el uso de recursos vegetales por las hormigas en un bosque montano bajo de Veracruz, México. *Acta Zoológica Mexicana* **73**, 45–55.