

## THE PATCHINESS OF EPIFOLIAR FUNGI IN TROPICAL FORESTS: HOST RANGE, HOST ABUNDANCE, AND ENVIRONMENT

GREGORY S. GILBERT,<sup>1,2,5</sup> DON R. REYNOLDS,<sup>3</sup> AND ARIADNA BETHANCOURT<sup>4</sup>

<sup>1</sup>*Environmental Studies, 1156 High Street, University of California, Santa Cruz, California 95064 USA*

<sup>2</sup>*Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Ancón, Panama*

<sup>3</sup>*Natural History Museum, 900 Exposition Boulevard, Los Angeles, California 90007 USA*

<sup>4</sup>*Departamento de Microbiología, Universidad de Panamá, Panama*

**Abstract.** Fungal symbioses affect the diversity, dynamics, and spatial patterns of trees in tropical forests. Their ecological importance is partly driven by their inherent patchiness. We used epifoliar fungi, a guild of common, benign, obligate, fungal symbionts of plants, as a model system to evaluate the relative importance of host phylogeny, host relative abundance, and microclimate on the three-dimensional distribution of plant–fungus symbioses. In parallel studies in rainforests in Panama and Australia, most epifoliar fungi were able to colonize several plant lineages but showed significant host preferences within the local plant community. More closely related plant species were not more likely to share fungal symbionts. Instead, fungal species were more likely to be shared by more abundant hosts, which supported a greater number and diversity of fungi. Environmental conditions strongly affected spatial distributions, with sites in the dark understory 2.5- to fourfold more likely to have epifoliar fungi than in the exposed forest canopy. In the understory, fungal incidence increased with canopy openness. Canopy trees supported only a subset of the fungal symbionts found in the understory, suggesting that adult trees are not reservoirs of these fungal symbionts for understory juveniles.

**Key words:** canopy access cranes; *Cleistanthus myrianthus*; epiphyll; phyloecology; plant–fungus symbiosis; Queensland Australia; San Lorenzo Panama; tropical rainforest canopy.

### INTRODUCTION

Fungal symbionts play key ecological and evolutionary roles in tropical forests. Ranging from mutualists to pathogens, symbionts affect individual plant performance, population dynamics, community structure, and diversity of plants (reviews in Saikkonen et al. 1998, Gilbert 2002, Allen et al. 2003). Ecological impacts are modulated by the patchiness of many plant–fungus symbioses, and the distribution and impacts of fungi depend on the biotic and abiotic environment of the interaction (Augsburger 1984, Gilbert et al. 1997, Kiers et al. 2000, Arnold and Herre 2003).

Host selectivity of fungal pathogens and mutualists is of central importance for many aspects of the biology of these interactions. For example, the importance of density- or distance-dependent transmission of plant pathogens will depend on the number of locally present hosts that can be infected and the spatial distribution of those hosts (Gilbert 2005). Studies of polypore fungi, leaf endophytes, and mycorrhizae suggest that polyphagy is probably the rule among tropical fungi, but that local host preferences and differential impacts are

common (Arnold et al. 2000, 2001, Kiers et al. 2000). Host specificity can be context dependent; fungi with broad host ranges may dominate diverse forests, while low-diversity forests are dominated by specialists (review in Gilbert 2005). Our understanding of host preferences, however, is in its infancy.

Spatial and temporal variation in plant–fungus symbioses in tropical forests can be generated by density- and distance-dependent infection processes as well as by microclimatic variation (Augsburger 1984, Gilbert et al. 1997, Arnold and Herre 2003, Kyllö et al. 2003). However, pathogens and mutualists could also directly affect the distribution or abundance of the hosts themselves; a virulent pathogen might locally eliminate populations of particular host species in precisely the areas where the pathogens have their greatest effects.

In order to decouple the effects of host distribution on fungi from those of fungi on host distribution, we need a model system in which the fungi are neutral commensals. Epifoliar fungi, or fungal epiphylls, comprise a polyphyletic guild of ascomycete fungi that obligately complete their entire life cycle on the surface of living plant leaves. Epifoliar fungi are functionally commensal with their host plants; they do not cause disease and in general plants can photo-compensate for the light lost to epiphyll cover (Anthony et al. 2002; but see Wood et al. 1988 for an exception). The several hundred known species of tropical epifoliar fungi employ one of several nutritional strategies and include superficial saprobes,

Manuscript received 5 August 2005; accepted 17 November 2005; final version received 17 January 2006. Corresponding Editor: D. R. Strong. For reprints of this Special Feature, see footnote 1, p. 539.

<sup>5</sup> E-mail: ggilbert@ucsc.edu

specialists on insect-produced honeydew, and weak parasites that obtain nutrition directly from the host plant (Reynolds and Gilbert 2005 and references within). The epifoliar fungi we have encountered come from diverse clades within the Ascomycetes (Lutzoni et al. 2004), and each lineage is comprised mainly of obligately epifoliar species found primarily on living leaves (Kirk et al. 2001). Epifoliar fungi are readily visible with the naked eye and have persistent structures useful for identification. They are widespread, show some degree of host preferences, and are affected by microclimatic variation (Barr 1987, Gilbert et al. 1997). These traits make epifoliar fungi useful for investigating the distribution of plant–fungal symbioses in tropical forests, without the complication of direct effects of the fungus on the host itself.

Here, we present epifoliar fungi as a novel system with which to ask critical questions about how host phylogeny, host relative abundance, and environmental conditions determine the distribution of tropical plant–fungal symbioses. We used canopy-access cranes in rainforests in Panama and Australia to assess the three-dimensional patterns of distribution of epifoliar fungi and to evaluate four underlying assumptions of many studies of plant–symbiont interactions in tropical forests: (1) obligate fungal symbionts (and other phytophages) in diverse tropical forests should be polyphagous (May 1991, Novotny et al. 2002), but their host range should be concentrated among closely related hosts (Webb et al. 2006); (2) hosts should be more likely to share fungi with common plant species than with rare ones; (3) environmental conditions shape the distribution and impacts of plant–fungal symbioses (Gillett 1962, Givnish 1999); and (4) adult trees are reservoirs for symbionts that infect nearby offspring (Janzen 1970, Connell 1971).

#### METHODS

We investigated the epifoliar fungi in tropical rainforests in Australia and Panama, using canopy-access construction cranes to access foliage from the top of the canopy to ground level. At each site we collected epifoliar fungi early in the rainy season (1) using radial transects to explore relative abundances and host ranges and (2) from a focal tree species in the canopy and understory to evaluate the likelihood that canopy trees act as fungal reservoirs for understory juveniles.

#### *Site descriptions*

The Australian Canopy Crane Research Facility (Stork and Cermak 2003), managed by the Rainforest Cooperative Research Centre, is located in lowland tropical rainforest at Cape Tribulation, near the Daintree National Park, North Queensland, Australia (16°04' S, 145°28' E; altitude 40 m). The vegetation is complex mesophyll vine forest with an irregular, storm-damaged canopy from 15 to 33 m tall. The site averages 3600 mm rain annually, with 70% usually falling

between December and April. A construction tower crane (Liebherr 91EC; Liebherr International AG, Bulle Switzerland), 47 m tall, with a radius of 55 m, provides full three-dimensional access to about 1.0 ha.

The San Lorenzo Canopy Crane, managed by the Smithsonian Tropical Research Institute, is located in lowland tropical rainforest on the San Lorenzo Protected Area on the Caribbean coast of the Republic of Panama (9°17' N, 79°58' W; altitude 130 m; Wright et al. 2003). The vegetation is tropical wet evergreen forest, with a canopy height of 35–45 m. The site averages 3152 mm rain annually. A Krøll K-700 crane (Kr II Cranes A/S, Nordkranvej, Denmark) has a 53-m reach that provides access to 0.9 ha of forest.

#### *Radial-transect surveys for host range and relative abundance*

We conducted systematic radial transects along the length of the crane reach, taking samples every 5 m, by collecting three leaves from all plant species within a 2-m radius. Leaves were not inspected for presence of fungi before collection. Parallel transects were run at the top of the canopy and at ground level directly below, providing estimates of the relative abundance of epifoliar fungi and their plant hosts and the ability to analyze both host range and spatial patterns of fungal incidence. At Cape Tribulation, we evaluated microclimate variation by estimating canopy openness using hemispherical digital images at each sample point in the canopy and understory (Appendix A). Fungi were identified based on morphological characters (methods and species list in Appendix B; Reynolds and Gilbert 2005, 2006).

We tested for host selectivity by calculating the probability that epifoliar fungi would never be found on a host species of a given relative frequency, assuming a binomial distribution under the null assumption that epifoliar fungi are host nonselective (Appendix C).

We used logistic regression to evaluate how phylogenetic distance and joint relative abundance (JRA) of hosts affected the probability that pairs of plant species would share taxa of epifoliar fungi. For all identifiable angiosperm plant species sampled in the radial transects, we used the Phylomatic component of Phylocom v3.34 (with the Phylomatic tree R20050610) to produce a tree topology based on the APG II backbone (Angiosperm Phylogeny Group 2003), and branch lengths set using Phylocom *blad*j function with ages from Wikstrom et al. (2001) (Phylocom v3.34 *available online*).<sup>6</sup> We then used the Phylocom *phydist* function to create a matrix of pairwise phylogenetic distances (in My) between each pair of plant taxa. We excluded samples from non-angiosperm hosts and samples for which the host could not be identified reliably at least to the family level. For each pair of plant species we calculated the joint relative

<sup>6</sup> (<http://www.phylodiversity.net/phylocom>)

TABLE 1. Epifoliar fungi collected in radial transects in the canopy and understory of the rain forest at San Lorenzo, Panama, and Cape Tribulation, Australia.

Stratum	No. sample sites (% with fungi)	No. plant samples (% with fungi)	No. plant species (% with fungi)	No. fungal collections	No. fungal species
Panama					
Canopy	72 (43.0%)	75 (16.1%)	31 (29.0%)	17	8
Understory	72 (16.7%)	489 (8.2%)	148 (18.24%)	54	21
Total	144 (29.9%)	564 (9.2%)	166 (20.5%)	71	23
Australia					
Canopy	120 (35.8%)	255 (4.3%)	68 (10.3%)	14	10
Understory	120 (9.2%)	588 (11.6%)	129 (27.1%)	95	24
Total	240 (22.5%)	843 (9.4%)	167 (23.9%)	109	25

abundance as the product of the proportion of all plant samples for those species (e.g., two species of 2% relative abundance each have a joint relative abundance =  $0.02 \times 0.02 = 0.0004$ ). We then used Proc Logistic (SAS, version 8.0; SAS Institute 2001) to select the best of all possible models using the independent variables phylogenetic distance, joint relative abundance (or  $\log_{10}$ [JRA]), and the interaction (Appendix D).

#### *Focal host survey: canopy-understory connection*

*Cleistanthus myrianthus* (Euphorbiaceae) is common in the Australian plot and was frequently colonized by epifoliar fungi both as mature canopy trees and as understory juveniles. We randomly selected 24 of the 73 mapped mature trees and determined whether they harbored epifoliar fungi by carefully examining (2 min with a hand lens) all reachable foliage (~2 m radius), at each of three different haphazardly chosen positions and levels of the tree crown. For all conspecific juveniles within 3 m of the trunk base (juvenile density), we inspected all leaves to determine if they were colonized by epifoliar fungi (20 adults and 50 juveniles total). Fungi collected from both the adults and juveniles were identified. Canopy openness at a point 1 m south of each canopy tree was estimated from the mean of four readings from a spherical densiometer taken at cardinal directions (1.3 m above the ground). We used logistic regression to test whether canopy openness, juvenile density, or the colonization status of the nearest canopy tree affected whether a juvenile was colonized by epifoliar fungi (Appendix E).

## RESULTS

### *Abundance and diversity of fungi and their hosts*

In the radial transects, epifoliar fungi were found on about 9% of the plant samples at each site (Table 1). This included 23 of 28 epifoliar species (Panama) and 25 of 35 species (Australia) found during extensive collections at the two sites (Reynolds and Gilbert 2005, 2006; D. R. Reynolds, G. S. Gilbert, and A. Bethancourt, *personal observation*), indicating a reasonably thorough fungal sampling (Appendix B).

Epifoliar fungi showed preferences among locally available plant species, being absent from 79.5% (Panama) and 76.1% (Australia) of all sampled plant species. Accounting for the rarity of hosts, this proportion was much higher than expected by chance if fungi had no host selectivity (Appendix C). For instance, in Panama, plant species of frequency 1, 2, 3, 4, or 5 in the collection (89% of all plant species at the site) were more often fungus-free than would be expected by chance ( $P = 0.015, 0.052, 0.039, 0.089, 0.067$ , respectively). In addition, even some very common hosts were unexpectedly free of epifoliar fungi (Australia, *Merremia peltata* [58 collections;  $P = 0.0003$ ]; Australia, *Calamus radicalis* [28 collections;  $P = 0.021$ ]; Panama, unidentified Fern 1 [23 collections,  $P = 0.045$ ]).

Diversity of epifoliar fungi and their host plants was similar in Panama and Australia (Appendix B). The 23 species of epifoliar fungi in Panama were found on 32 identifiable vascular plant species (plus 4 unidentified), from at least 29 genera, 22 families, and 17 orders. Similarly, the 25 fungal species found in radial transects in Australia were distributed across 40 plants species from at least 27 genera, 19 families, and 17 orders.

### *Phylogeny and relative abundance in host range determination*

Although epifoliar fungi clearly show preferences for some plant species over others, we found no evidence for the expected phylogenetic signal in host range. Epifoliar fungi were polyphagous: all but one fungal species collected three or more times were found on multiple plant orders, and at neither site was the phylogenetic distance between plant species predictive of the probability of sharing fungal symbionts (Appendix D). In contrast, more common plants were much more likely to share fungal species than were rare plants. At both sites, the logarithm of the joint relative abundance of host plants was a strong indicator of the likelihood of sharing fungal symbionts, correctly predicting 63% (Panama) and 71% (Australia) of fungal sharing between plant species (Fig. 1 and Appendix D). Greater sharing may be a function of abundant plants being more likely to

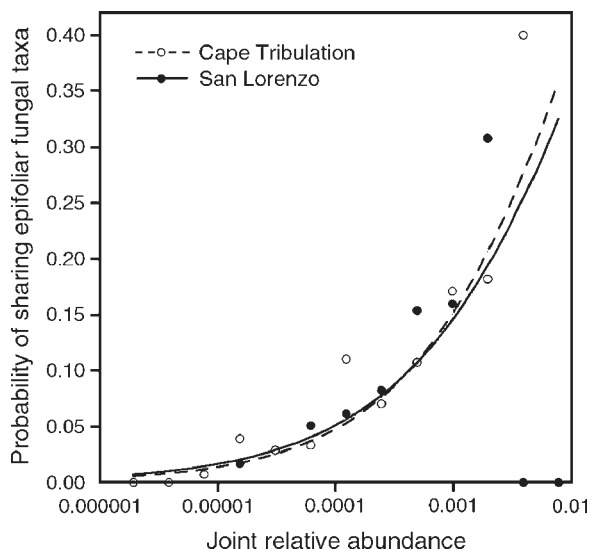


FIG. 1. The probability that two plant species share epifoliar fungal symbionts in the rain forest at Cape Tribulation, Australia, and San Lorenzo, Panama. Points indicate the proportion of host species pairs that shared at least one fungal taxon at that binned joint relative abundance (JRA). Lines are the predicted probabilities from best-fit logistic regression models (Appendix D). For each line,  $p(\text{sharing}) = e^y / (1 + e^y)$  where  $y = 2.0995 + 1.2722 \log_{10}(\text{JRA})$  for Panama and  $y = 1.7059 + 1.1563 \log_{10}(\text{JRA})$  for Australia.

encounter compatible fungi under appropriate conditions.

#### Canopy–understory distribution of epifoliar fungi

Epifoliar fungi were much more common and diverse at sample sites in the understory than in the canopy (Panama, 43.0% vs. 16.7%, respectively, Fisher's exact test  $P = 0.0009$ ; Australia, 35.8% vs. 9.2%,  $P \leq 0.0001$ ) (Table 1). Similarly, in Australia, the proportion of understory plant samples with fungi was twice that in the canopy. However, in Panama there were many fewer host species in the canopy, and because rare species in the understory tended to be free of fungi, the pattern there was reversed (Table 1). This difference was largely due to the much more dominant presence of climber species and greater plant diversity in the Australian canopy.

All but three of the 18 fungal species found in the canopy (both sites combined) were also recorded in the understory (Appendix B), suggesting that the canopy mycota is a nested subset of that in the understory. The three canopy-only species were each found only once or twice.

In only one case (both sites combined), was the same fungal species found in both the canopy sample point and the understory sample point directly below. This lack of fungal connection between strata may reflect the differences in composition of potential plant hosts in the canopy and understory. Although the forest canopy has a high density of leaves, the plant diversity per sample

site was much less than in the understory (Panama, canopy  $1.04 \pm 0.37$  species vs. understory  $6.69 \pm 2.47$  species [mean  $\pm$  SD], paired  $t = 18.25$ ,  $df = 71$ ,  $P \leq 0.0001$ ; Australia, canopy  $2.14 \pm 1.17$  vs. understory  $4.91 \pm 2.07$ , paired  $t = 12.52$ ,  $df = 119$ ,  $P \leq 0.0001$ ). Few plant species were shared between canopy and understory (Panama, 10 of 92 species, 10.9%; Australia, 30 of 167 species, 17.9%).

In addition to host distribution, environmental conditions differ between the canopy and understory (Appendix A), and may limit the distribution of different species of fungi to one stratum or the other. To test the effect of microclimate on the patchiness of fungal symbioses and explore the potential of mature trees as fungal reservoirs, we studied fungi associated with a single common host.

#### Focal host: canopy–understory connection

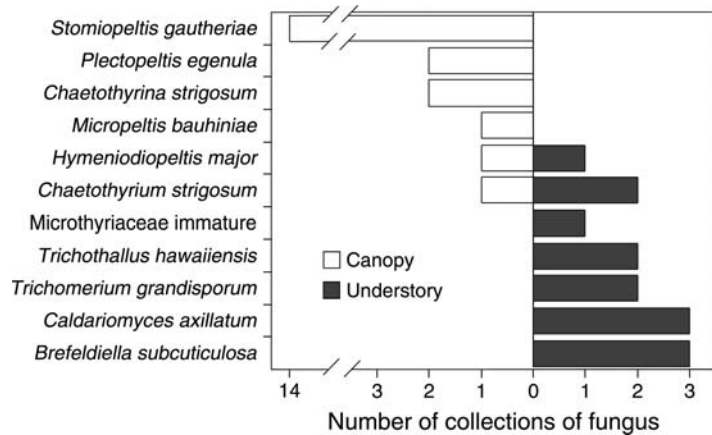
*Cleistanthus myrianthus* is an unusually good host for epifoliar fungi, representing 38% of the fungal collections and half of the fungal species recorded from the Australian canopy, with less than 3% of the leaf collections. Fungi were found on 75% of canopy adults and 28% of understory juveniles. Logistic regression indicated that neither juvenile density nor the colonization status of the nearest adult significantly affected the probability of finding epifoliar fungi on a juvenile (Appendix E). However, juveniles in more open environments were much more likely to have epifoliar symbionts. The regression  $y = -2.8624 + 0.2729(\text{canopy openness})$ , where  $p(\text{juvenile colonized}) = e^y / (1 + e^y)$  (AIC = 54.48,  $\chi^2 = 8.82$ ,  $P = 0.003$ ), correctly predicted which juveniles were colonized by epifoliar fungi 72.6% of the time.

Of the 12 fungal species found on *Cleistanthus myrianthus*, there was little overlap between the adult crown and understory juveniles (Fig. 2). Canopy foliage was more frequently colonized by epifoliar fungi, but species richness was much lower, dominated by *Stomiopeltis gautheriae*, a canopy specialist (Fisher's exact test,  $P \leq 0.0001$ ). In fact, only two fungal species were found in both strata, and neither were on the canopy tree above the understory collections. All species except *Hymeniodiopeltis major* were found on other hosts (Reynolds and Gilbert 2005); the fungi of *C. myrianthus* appear to be stratum specialists rather than host specialists.

#### DISCUSSION

Epifoliar fungi represent a novel and tractable system for investigating a range of questions about host range, host abundance, and environmental influences on the patchiness of plant–fungus symbioses. Their broad phylogenetic distribution across Ascomycetes and their close but commensal association with living plant hosts made them excellent candidates to address several underlying assumptions in research on the ecology of fungal symbionts of tropical trees.

FIG. 2. Distribution of epifoliar fungal symbionts in the canopy (open bars) and understory (solid bars) on *Cleistanthus myrianthus* (Euphorbiaceae) in Cape Tribulation, Australia.



First, because the rarity of most tree species in tropical forests makes reliable encounter difficult, fungal symbionts in diverse rainforests should be polyphagous (May 1991). However, the complexity of interactions between plant and fungal symbionts means extreme generalism is also unlikely. It is often assumed that fungi are more likely to share closely related hosts, so that the probability of sharing hosts declines with increasing phylogenetic distance between hosts (Webb et al. 2006). For instance, host-range testing for the safety of potential bioherbicide pathogens is based largely on the centrifugal phylogenetic method of host selection (Wapshere 1974, Briese 2003). For epifoliar fungi, we found that fungi were polyphagous, colonizing hosts from a diversity of plant lineages, but that contrary to expectation we detected no phylogenetic signal in the observed host range. Whether more intimate pathogenic or mutualistic symbioses show phylogenetic signal needs to be rigorously tested.

Second, common plant species were more likely to harbor and share fungal species than were rare species, likely a function of common hosts more efficiently sampling the local mycota. If we assume a neutral model that fungi are host non-selective and able to colonize any host species encountered in an appropriate environment, then more common hosts will simply accumulate a greater richness of fungi as a simple species-area relationship. However, epifoliar fungi show host preferences, suggesting the possibility that common hosts “culture” their own symbionts in a density-dependent manner, or that other density-dependent agents (such as honeydew secreting insects) indirectly facilitate the growth of epifoliar fungi. In either case, propagules dispersing from those hosts can colonize suitable heterospecific hosts, and the likelihood that a particular tree species would encounter such spillover (Power and Mitchell 2004) should be proportional to its local abundance. Suitable common hosts could then provide additional density-dependent feedback, increasing the relative abundance of symbionts that share common hosts. In addition to opportunities of encountering hosts to which the fungus is pre-adapted, common hosts

provide greater opportunities for the evolution of novel host–fungus associations (Parker and Gilbert 2004).

Third, environmental conditions have a strong controlling influence over the distribution and impacts of plant–fungal symbioses (Gillett 1962, Givnish 1999). We found strong vertical stratification for both overall abundance of epifoliar fungi and for particular fungal species. Vertical stratification is common in foliar plant pathogens and many other tropical taxa (Gilbert 1995, DeVries et al. 1997, Gilbert et al. 1997, Basset et al. 2001, García-Guzmán and Dirzo 2004). In the horizontal dimension, variation in canopy openness was a very strong predictor of the distribution of epifoliar fungi in the forest understory, as shown earlier for the epifoliar *Scolecopeltidium* (Gilbert et al. 1997). The patchiness of plant–fungal symbioses is clearly driven by environmental impacts on fungal growth as well as by the availability of suitable host species.

Finally, we found no evidence that adult trees act as reservoirs for fungal symbionts to infect nearby off-springs, one of the assumptions of the Janzen–Connell hypothesis (Janzen 1970, Connell 1971). For insects, Basset et al. (2001) found little evidence for exchange between canopy and understory for insects, and suggested canopy insects are likely resident habitat specialists, although Barone (2000) found that adults and juveniles of two rainforest tree species largely shared their suites of chewing herbivores. In our study, understory juveniles encountered a much more diverse suite of fungal symbionts than did their mature counterparts. This canopy–understory disconnection is important because although several studies have shown distance-dependent pathogen or mutualist patterns for soilborne organisms (Augspurger 1984, Newbery et al. 2000, Hood et al. 2004), to our knowledge none have yet demonstrated that the canopy serves as a reservoir for symbionts. Density of airborne fungal spores is many times lower in the rainforest canopy than the understory (Gilbert and Reynolds 2005), probably reflecting both reduced colonization of canopy leaves and environmental conditions hostile to spore production. Our data suggest that the dynamics of foliar fungal symbioses

with above-ground plant parts in the rainforest understory may be largely disconnected from dynamics in the canopy above.

## ACKNOWLEDGMENTS

We thank the Andrew W. Mellon Foundation for funding, and R. Cooper, M. Cermak, N. Stork, S. J. Wright, R. Rader, B. Howlett, S. Aguilar, R. Perez, the Australian Canopy Crane Research Facility, and the Smithsonian Tropical Research Institute for facilitating the work. We thank C. O. Webb and I. M. Parker for helpful discussions or comments on the manuscript. Additional support was provided to GSG from the National Science Foundation DEB 0515520.

## LITERATURE CITED

- Allen, M. F., W. Swenson, J. I. Querejeta, L. M. Egerton-Warburton, and K. K. Treseder. 2003. Ecology of mycorrhizae: A conceptual framework for complex interactions among plants and fungi. *Annual Review of Phytopathology* 41:271–303.
- Angiosperm Phylogeny Group. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141:399–436.
- Anthony, P. A., J. A. M. Holtum, and B. R. Jackes. 2002. Shade acclimation of rainforest leaves to colonization by lichens. *Functional Ecology* 16:808–816.
- Arnold, A. E., and E. A. Herre. 2003. Canopy cover and leaf age affect colonization by tropical fungal endophytes: ecological pattern and process in *Theobroma cacao* (Malvaceae). *Mycologia* 95:388–398.
- Arnold, A. E., Z. Maynard, and G. S. Gilbert. 2001. Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. *Mycological Research* 105:1502–1507.
- Arnold, A. E., Z. Maynard, G. S. Gilbert, P. D. Coley, and T. A. Kursar. 2000. Are tropical fungal endophytes hyperdiverse? *Ecology Letters* 3:267–274.
- Augsburger, C. K. 1984. Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. *Ecology* 65:1705–1712.
- Barone, J. A. 2000. Comparison of herbivores and herbivory in the canopy and understory for two tropical tree species. *Biotropica* 32:307–317.
- Barr, M. E. 1987. *Podromus to Loculoascomycetes*. M. E. Barr, Amherst, Massachusetts, USA.
- Basset, Y., et al. 2001. Stratification and diel activity of arthropods in a lowland rainforest in Gabon. *Biological Journal of the Linnean Society* 72:585–607.
- Briese, D. T. 2003. The centrifugal phylogenetic method used to select plants for host-specificity testing of weed biological control agents: Can and should it be modernised? Pages 23–33 in H. S. Jacob and D. T. Briese, editors. *Improving the selection, testing and evaluation of weed biological control agents*. Technical Series #7. CRC for Australian Weed Management, Glen Osmond, Australia.
- Connell, J. H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rainforest trees. Pages 298–312 in P. J. Boer and G. R. Graadwell, editors. *Dynamics of numbers in populations*. Proceedings of the Advanced Study Institute, Osterbeek 1970. Centre for Agricultural Publication and Documentation, Wageningen, The Netherlands.
- DeVries, P. J., D. Murray, and R. Lande. 1997. Species diversity in vertical, horizontal, and temporal dimensions of a fruit-feeding butterfly community in an Ecuadorian rainforest. *Biological Journal of the Linnean Society* 62:343–364.
- García-Guzmán, G., and R. Dirzo. 2004. Incidence of leaf pathogens in the canopy of a Mexican tropical wet forest. *Plant Ecology* 172:31–50.
- Gilbert, G. S. 1995. Rain forest plant diseases: the canopy–understory connection. *Selbyana* 16:75–77.
- Gilbert, G. S. 2002. Evolutionary ecology of plant diseases in natural systems. *Annual Review of Phytopathology* 40:13–43.
- Gilbert, G. S. 2005. The dimensions of plant disease in tropical forests. Their role in the maintenance of species diversity. Pages 141–164 in D. R. F. P. Burslem, M. A. Pinard, and S. Hartley, editors. *Biotic interactions in the tropics*. Cambridge University Press, Cambridge, UK.
- Gilbert, G. S., and D. R. Reynolds. 2005. Nocturnal fungi: airborne spores in the canopy and understory of a tropical rain forest. *Biotropica* 37:461–463.
- Gilbert, G. S., N. Talaro, C. A. Howell, and A. Symstad. 1997. Multiple-scale spatial distribution of the fungal epiphyll *Scoleopeltidium* on *Trichilia* spp. in two lowland moist tropical forests. *Canadian Journal of Botany* 75:2158–2164.
- Gillett, J. B. 1962. Pest pressure, an underestimated factor in evolution. *Systematics Association Publication Number* 4: 37–46.
- Givnish, T. J. 1999. On the causes of gradients in tropical tree diversity. *Journal of Ecology* 87:193–210.
- Hood, L. A., M. D. Swaine, and P. A. Mason. 2004. The influence of spatial patterns of damping-off disease and arbuscular mycorrhizal colonization on tree seedling establishment in Ghanaian tropical forest soil. *Journal of Ecology* 92:816–823.
- Janzen, D. H. 1970. Herbivores and the number of tree species in tropical forests. *American Naturalist* 104:501–527.
- Kiers, E. T., C. E. Lovelock, E. L. Krueger, and E. A. Herre. 2000. Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. *Ecology Letters* 3: 106–113.
- Kirk, P. M., P. F. Cannon, J. C. David, and J. A. Stalpers. 2001. *Ainsworth and Bisby's dictionary of the fungi*. Ninth edition. CABI Bioscience, Egham, Surrey, UK.
- Kyllo, D. A., V. Velez, and M. T. Tyree. 2003. Combined effects of arbuscular mycorrhizas and light on water uptake of the neotropical understory shrubs, *Piper* and *Psychotria*. *New Phytologist* 160:443–454.
- Lutzoni, F., et al. 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* 91:1446–1480.
- May, R. M. 1991. A fondness for fungi. *Nature* 352:475–476.
- Newbery, D. M., I. J. Alexander, and J. A. Rother. 2000. Does proximity to conspecific adults influence the establishment of ectomycorrhizal trees in rain forest? *New Phytologist* 147: 401–409.
- Novotny, V., Y. Basset, S. E. Miller, G. D. Weiblen, B. Bremer, L. Cizek, and P. Drozd. 2002. Low host specificity of herbivorous insects in a tropical forest. *Nature* 416:841–844.
- Parker, I. M., and G. S. Gilbert. 2004. The evolutionary ecology of novel plant–pathogen interactions. *Annual Review of Ecology, Evolution, and Systematics* 35:675–700.
- Power, A. G., and C. E. Mitchell. 2004. Pathogen spillover in disease epidemics. *American Naturalist* 164:S79–S89.
- Reynolds, D. R., and G. S. Gilbert. 2005. Epifoliar fungi from Queensland, Australia. *Australian Systematic Botany* 18: 265–289.
- Reynolds, D. R., and G. S. Gilbert. 2006. Epifoliar fungi from Panama. *Cryptogamie Mycologie* 27:249–270.
- Saikkonen, K., S. H. Faeth, M. Helander, and T. J. Sullivan. 1998. Fungal endophytes: a continuum of interactions with host plants. *Annual Review of Ecology and Systematics*. 29: 319–343.
- SAS Institute. 2001. SAS 8.0. SAS Institute, Cary, North Carolina, USA.
- Stork, N. E., and M. Cermak. 2003. Australian canopy crane: getting on top of the world's last biological frontier. Pages

- 108–114 in Y. Basset, V. Horlyck, and S. J. Wright, editors. Studying forest canopies from above: the international canopy crane network. Smithsonian Tropical Research Institute, Balboa, Ancón, Panamá, Panama.
- Wapshere, A. I. 1974. A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* 77:20–211.
- Webb, C. O., G. S. Gilbert, and M. J. Donoghue. 2006. Phylodiversity dependent seedling mortality, size structure, and disease in a Bornean rain forest. *Ecology* 87(Supplement): S123–S131.
- Wikstrom, N., V. Savolainen, and M. W. Chase. 2001. Evolution of angiosperms: calibrating the family tree. *Proceedings of the Royal Society, Series B* 268:2211–2220.
- Wood, B. W., W. L. Tedders, and C. C. Reilly. 1988. Sooty mold fungus on pecan foliage suppresses light penetration and net photosynthesis. *HortScience* 23:851–853.
- Wright, S. J., et al. 2003. Tropical canopy biology program, Republic of Panama. Pages 136–155 in Y. Basset, V. Horlyck, and S. J. Wright, editors. Studying forest canopies from above: the international canopy crane network. Smithsonian Tropical Research Institute, Balboa, Ancón, Panamá, Panama.

#### APPENDIX A

Canopy openness as a measure of microclimate at canopy and understory sample sites for radial transects in Cape Tribulation, Australia (*Ecological Archives* E088-035-A1).

#### APPENDIX B

Identification and frequency of collection of epifoliar fungi in canopy or understory radial transects from Cape Tribulation, Australia, or San Lorenzo, Panama (*Ecological Archives* E088-035-A2).

#### APPENDIX C

Calculation of the probability of plant species being free of epifoliar fungi, under the assumption that fungi are host nonselective (*Ecological Archives* E088-035-A3).

#### APPENDIX D

Logistic regression results for the effect of phylogenetic distance (My), joint relative abundance (JRA), or the interaction (MyxJRA) on the probability that two plant species shared epifoliar fungal symbionts, from radial transects at San Lorenzo, Panama, and Cape Tribulation, Australia (*Ecological Archives* E088-035-A4).

#### APPENDIX E

Logistic regression results for the effect of canopy fungi (presence/absence of fungi on nearest mature conspecific), juvenile density (number of juveniles in 3-m radius around adult), and percentage of canopy openness on the proportion of juvenile *Cleistanthus myrianthus* colonized by epifoliar fungi at Cape Tribulation, Australia (*Ecological Archives* E088-035-A5).

**Gregory S. Gilbert, Don R. Reynolds, and Ariadna Bethancourt. 2007. The patchiness of epifoliar fungi in tropical forests: host range, host abundance, and environment. *Ecology* 88:575–581.**

Appendix A. Canopy openness as a measure of microclimate at canopy and understory sample sites for radial transects in Cape Tribulation, Australia.

The openness of the forest canopy provides an integrated measure of variation in irradiation, relative humidity, and temperature. Canopy openness was estimated at each sample point along radial transects in the canopy (using a Nikon fisheye lens camera) and in the understory with a CID-110 Canopy Analyzer (CID Inc., Camas, Washington, USA). Due to failure of the CID-110, hemispherical images could not be collected with the same instruments in both strata. All images were collected under conditions of uniform lighting (early or late in day, overcast conditions). Percent canopy openness was calculated from the digital images using Canopy Analyzer software from CID, Inc.

Light conditions were highly variable across sample points, and distribution of light environments in the canopy and understory were largely non overlapping (Fig. A1).

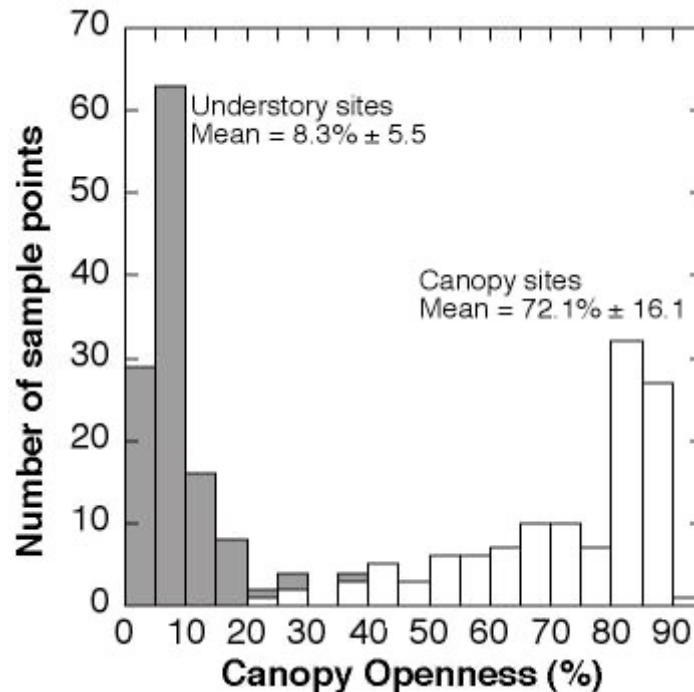


FIG. A1. Distribution of canopy openness in the canopy and understory of radial transect sample points at Cape Tribulation, Australia.

---

*Ecological Archives* E088-035-A2

**Gregory S. Gilbert, Don R. Reynolds, and Ariadna Bethancourt. 2007. The patchiness of epifoliar fungi in tropical forests: host range, host abundance, and environment. *Ecology* 88:575–581.**

Appendix B. Identification and frequency of collection of epifoliar fungi in canopy or understory radial transects from Cape Tribulation, Australia, or San Lorenzo, Republic of Panama.

Fungi were identified by D. Reynolds based on morphological characteristics, through examination of fungi from pressed and dried leaves. Observations and measurements of fungal structures were made from dried specimens mounted in lactophenol and examined using Zeiss light microscopes (Carl Zeiss International, Göttingen, Germany) and with a Cambridge scanning electron microscope (Hitachi S-3000N, Hitachi Scientific Instruments, Nitachinaka, Japan). Specimens were compared to type and other collections from world-wide herbaria and from descriptions in relevant literature (Reynolds and Gilbert 2005)(Reynolds, Gilbert, and Bethancourt, *unpublished data*). Specimens from both sites are deposited at the University Herbarium (UC) at the University of California, Berkeley, USA, and from Panama at the Herbarium of the University of Panama.

<b>Cape Tribulation, Australia</b>					
Family	Habit	Fungus	Canopy	Understory	Total
Antennulariaceae	saprobic	<i>Antennulariella californica</i>	0	1	1
Asterinaceae	biotrophic	<i>Asterina eupomatiae</i>	1	11	12
Asterinaceae	biotrophic	<i>Asterina radulafistula</i>	0	1	1
mitotic ascomycete		<i>Asterostomella stophani</i>	0	4	4
Brefeldiaceae	biotrophic	<i>Brefeldiella subcuticulosa</i>	4	16	20
Capnodiaceae	saprobic, honeydew	<i>Caldariomyces axillatum</i>	1	14	15
Micropeltidiaceae	saprobic	<i>Chaetothyriina costaricensis</i>	1	2	3
Chaetothyriaceae	saprobic	<i>Chaetothyrium fusisporum</i>	0	1	1
Chaetothyriaceae	saprobic	<i>Chaetothyrium strigosum</i>	0	3	3
Capnodiaceae	saprobic, honeydew	<i>Conidiocarpus philippensis</i>	0	1	1
mitotic Ascomycete	saprobic	<i>Elachopeltis andinas</i>	0	1	1
mitotic Ascomycete	saprobic	<i>Enthallopycnidium gouldiae</i>	0	2	2
mitotic Ascomycete	saprobic	<i>Hymeniodiopeltis major</i>	1	1	2
Meliolaceae	biotrophic	<i>Meliola alstoniae</i>	1	2	3
Micropelidaceae	superficial	<i>Micropeltis bambusina</i>	0	7	7

Micropelidaceae	saprobic	<i>Micropeltis bauhiniae</i>	1	6	7
Micropeltidiaceae	saprobic	<i>Micropeltis sp.</i>	0	3	3
mitotic Ascomycete	saprobic	<i>Parastigmatellina asiatica</i>	1	1	2
mitotic Ascomycete	saprobic	<i>Plectopeltis egenula</i>	0	2	2
Schizothyriaceae	saprobic	<i>Plochrompeltis ellisii</i>	1	1	2
mitotic Ascomycete	saprobic	<i>Sporidesmium macrurum</i>	0	4	4
Micropeltidiaceae	saprobic	<i>Stigmatodothis palawanensis</i>	0	1	1
Micropelticiaceae	saprobic	<i>Stomiopeltis gautheriae</i>	2	0	2
Capnodiaceae	saprobic	<i>Trichomerium grandisporum</i>	0	8	8
Antennulariaceae	saprobic	<i>Trichothallus hawaiiensis</i>	0	2	2
		Grand Total	14	95	109

<b>San Lorenzo, Panama</b>					
Family	Habit	Fungus	Canopy	Understory	Total
Incertae sedis	saprobic	<i>Fumiglobus ficina</i>	0	1	1
Brefeldiellaceae	biotrophic	<i>Brefeldiella brasiliensis</i>	3	5	8
Chaetothyriaceae	saprobic	<i>Ciferriusia orientalis</i>	0	2	2
Capnodiaceae	saprobic	<i>Conidiocarpus penzigii</i>	0	1	1
Saccardiaceae	saprobic	<i>Cyanodiscus glabrescens</i>	0	6	6
mitotic Ascomycete	saprobic	<i>Elachopeltella rubescens</i>	1	2	3
mitotic Ascomycete	saprobic	<i>Hansfordiopeltopsis amazonensis</i>	2	5	7
Cocconiaceae	saprobic	<i>Limacinula tenuis</i>	1	0	1
Meliolaceae	biotrophic	<i>Meliola protii</i>	0	1	1
Schizothyriaceae	saprobic	<i>Metathyriella roupalae</i>	0	3	3
Micropeltinaceae	saprobic	<i>Micropeltis bambusina</i>	0	5	5
Micropeltinaceae	saprobic	<i>Micropeltis borgorensis</i>	0	1	1
Micropeltinaceae	saprobic	<i>Micropeltis caesalpiniae</i>	0	2	2
Micropeltinaceae	saprobic	<i>Micropeltis clavispora</i>	1	0	1
Micropeltinaceae	saprobic	<i>Micropeltis megasperma</i>	0	1	1
Micropeltinaceae	saprobic	<i>Micropeltis aspidiospermae</i>	0	1	1
Micropeltinaceae	saprobic	<i>Micropeltis sp1</i>	0	3	3
Schizothyriaceae	saprobic	<i>Metathyriella roupalae</i>	1	3	4
Schizothyriaceae	saprobic	<i>Plochrompeltis ellisii</i>	0	1	1
Schizothyriaceae	saprobic	<i>Schizothyriaceae</i> immature	6	6	12
		Sterile tufts	0	1	1

Vizellaceae		<i>Vizella royenae</i>	2	4	6
		Grand Total	17	54	71

LITERATURE CITED

Reynolds, D. R., and G. S. Gilbert. 2005. Epifoliar fungi from Queensland, Australia. *Australian Systematic Botany* **18**:265–289.

---

[\[Back to E088-035\]](#)

**Gregory S. Gilbert, Don R. Reynolds, and Ariadna Bethancourt. 2007. The patchiness of epifoliar fungi in tropical forests: host range, host abundance, and environment. *Ecology* 88:575–581.**

Appendix C. Calculation of the probability of plant species being free of epifoliar fungi, under the assumption that fungi are host non-selective.

In order to calculate the probability that plant species of particular abundance would be fungus-free (or have any particular number of fungal collections), we used the binomial distribution as presented in Sokal and Rohlf (1995: pp.71–74), where:

$$\left(\frac{k}{Y}\right) p^Y q^{k-Y} = \frac{k!}{Y!(k-Y)!} p^Y (1-p)^{k-Y} \quad (C.1)$$

where:

k = number of collections of a plant species

Y = number of those collections with epifoliar fungi,

p = proportion of all plant samples that had epifoliar fungi, and

q = 1-p = proportion of all samples with no fungi

For Cape Tribulation  $p = 109/843 = 0.129$  and for San Lorenzo  $p = 71/564 = 0.126$ .

For each observed frequency of a plant species at each site, we calculated the probability of observing zero fungi – p(NoFungi) – on a plant species of that frequency.

For each frequency, we then tallied the number of observed species with or without epifoliar fungi. We then used p(NoFungi) in formula (1) to calculate the probability that, under the assumption of host non-selectivity, the observed proportion of plant species of a particular frequency would be free of epifoliar fungi (Fig. C1).

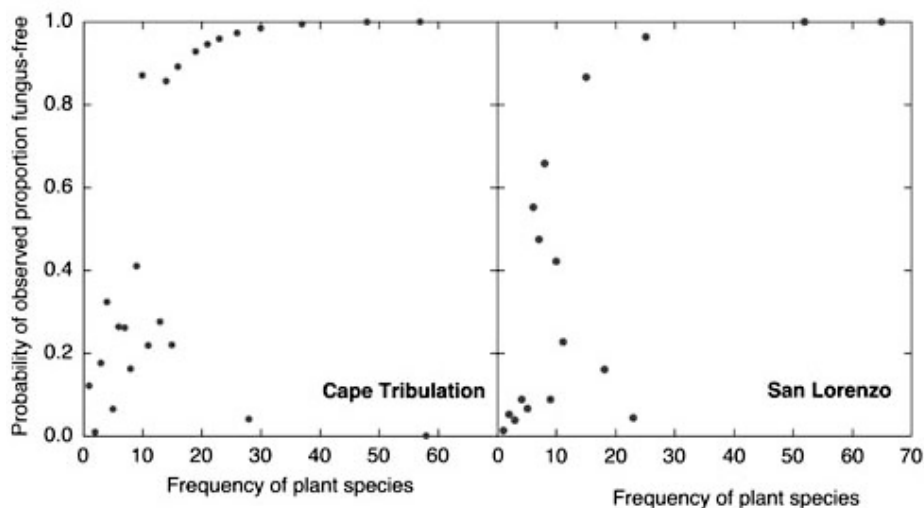


FIG. C1. For both Cape Tribulation, Australia and San Lorenzo, Panama, we observed many more fungus-free species than would be expected by chance under the assumption that fungi are not host selective, as expected if there was local host selectivity. In particular, less common species were less likely to harbor epifoliar fungi than expected by chance.

Probabilities of being fungus free ( $p(\text{NoFungi})$ ) for a particular species of given frequency, and of the probability of finding the observed number of fungus-free species of that frequency, as used in Fig. C1.

**For Cape Tribulation, Australia**

Species frequency	$p(\text{NoFungi})$	Obs. spp. without fungi	Obs. spp. with fungi	Total no. spp.	$p(\text{that many w/o fungi})$
1	0.8707	57	10	67	0.121042
2	0.758118	34	3	37	0.00896
3	0.660094	13	5	18	0.175609
4	0.574743	3	1	4	0.322948
5	0.50429	4	0	4	0.064673
6	0.435723	3	2	5	0.263401
7	0.379384	3	3	6	0.261058
8	0.33033	3	2	5	0.161646
9	0.287618	1	1	2	0.409788
10	0.8707	1	0	1	0.8707
11	0.218049	1	0	1	0.218049
13	0.165307	1	1	2	0.275961
14	0.143932	0	1	1	0.856068
15	0.125322	1	1	2	0.219233
16	0.109118	0	1	1	0.890882
19	0.072028	0	1	1	0.927972
21	0.054606	0	1	1	0.945394
23	0.041398	0	1	1	0.958602
26	0.027326	0	1	1	0.972674
28	0.020717	1	1	2	0.040575
30	0.015706	0	1	1	0.984294
37	0.005958	0	1	1	0.994042
48	0.001299	0	1	1	0.998701
57	0.000374	0	1	1	0.999626
58	0.000325	1	0	1	0.000325

**For San Lorenzo, Panama**

Species frequency	$p(\text{NoFungi})$	Obs. spp. without fungi	Obs. spp. with fungi	Total no. spp.	$p(\text{that many w/o fungi})$
1	0.874113	85	5	90	0.015001

2	0.764074	25	3	28	0.05153
3	0.667888	9	10	19	0.039879
4	0.58381	2	5	7	0.089376
5	0.510316	4	0	4	0.06782
6	0.446074	0	1	1	0.553926
7	0.389919	1	1	2	0.475764
8	0.340834	0	1	1	0.659166
9	0.297927	2	0	2	0.088761
10	0.260422	1	3	4	0.421395
11	0.227639	1	0	1	0.227639
15	0.132898	0	1	1	0.867102
18	0.088761	1	1	2	0.161764
23	0.045296	1	0	1	0.045296
25	0.03461	0	1	1	0.96539
52	0.000915	0	1	1	0.999085
65	0.000159	0	1	1	0.999841

LITERATURE CITED

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. Third Edition. W. H. Freeman, New York, New York, USA.

---

[\[Back to E088-035\]](#)

**Gregory S. Gilbert, Don R. Reynolds, and Ariadna Bethancourt. 2007. The patchiness of epifoliar fungi in tropical forests: host range, host abundance, and environment. *Ecology* 88:575–581.**

Appendix D. Logistic regression results for the effect of phylogenetic distance (My), joint relative abundance (JRA), or the interaction (MyxJRA) on the probability that two plant species shared epifoliar fungal symbionts, from radial transects at San Lorenzo, Panama and Cape Tribulation, Australia.

Akaike Information Criterion (AIC), Likelihood Ratio (LR), *P* values of Wald test chi-square estimates for each factor, and percent concordance were used to select the best model for each site. At neither site did phylogenetic distance between plant species pairs contribute significantly to the model. Intercept was always significant ( $P \leq 0.004$  for all).

Site	Model	AIC	LR	<i>P</i> values				Conc.%
				LR	My	JRA	MyxJRA	
SanLorenzo	1	787.4	18.7	0.0003	0.5426	0.0067	0.0532	61.4
SanLorenzo	2	789.3	14.8	0.0006	0.6816	0.0001		60.8
SanLorenzo	3	802	0.1	0.8379	0.8374			23.3
SanLorenzo	4	787.4	14.67	0.0001		0.0001		60.4
SanLorenzo	5	762.7	39.4	0.0001		0.0001†		63.3
CapeTrib	1	796	27.2	0.0001	0.5328	0.1944	0.9277	63.6
CapeTrib	2	794	27.2	0.0001	0.4079	0.0001		63.7
CapeTrib	3	818.3	0.9	0.3407	0.3293			34.8
CapeTrib	4	792.7	26.6	0.0001		0.0001		62.7
CapeTrib	5	754.7	64.5	0.0001		0.0001†		71.4

†Model uses log<sub>10</sub>(joint relative abundance).

At both sites, model 5 was the best, taking the form  $p(\text{sharing}) = \text{expy} / (1 + \text{expy})$  where:

$$y = 2.0995 + 1.2722 \log_{10} \text{JRA (for San Lorenzo)}$$

$$y = 1.7059 + 1.1563 \log_{10} \text{JRA (for Cape Tribulation)}$$

To compare the logistic model predictions to the rates of fungal sharing observed at each site (Fig. 1), we grouped all pairwise joint relative abundances into log-series bins with lower bounds at 0.5x. In each bin, we calculated the proportion of plant pairs that shared fungal taxa.

	San Lorenzo, Panama	Cape Tribulation, Australia
--	---------------------	-----------------------------

$x$	Lower bound	No. shared pairs	No. total pairs	Proportion shared	No. shared pairs	No. total pairs	Proportion shared
19	1.91E-06	0	0		0	55	2.85E-06
18	3.82E-06	0	0		0	110	5.71E-06
17	7.63E-06	0	0		1	133	3.42E-05
16	1.53E-05	6	351	0.0171	10	255	0.0001
15	3.05E-05	0	0		9	311	0.0005
14	6.10E-05	11	216	0.0509	9	271	0.0014
13	1.22E-04	42	683	0.0615	28	254	0.0048
12	2.44E-04	17	206	0.0825	14	199	0.0127
11	4.88E-04	20	130	0.1538	13	121	0.0308
10	9.77E-04	8	50	0.1600	13	76	0.0455
9	1.95E-03	4	13	0.3077	6	33	0.0634
8	3.91E-03	0	3	0.0000	4	10	0.0395
7	7.81E-03	0	1	0.0000	0	2	0.0174

[\[Back to E088-035\]](#)

**Gregory S. Gilbert, Don R. Reynolds, and Ariadna Bethancourt. 2007. The patchiness of epifoliar fungi in tropical forests: host range, host abundance, and environment. *Ecology* 88:575–581.**

Appendix E. Logistic regression results for the effect of canopy fungi (presence/absence of fungi on nearest mature conspecific), juvenile density (no. juveniles in 3-m radius around adult), and percentage canopy openness on the proportion of juvenile *Cleistanthus myrianthus* colonized by epifoliar fungi at Cape Tribulation, Australia.

Akaike Information Criterion (AIC), Likelihood Ratio (LR), *P* values of Wald test chi-square estimates for each factor, and percentage concordance were used to select the best model. Intercept was always significant ( $P \leq 0.042$  for all).

Model	AIC	LR Chi-square	LR	<i>P</i> values			Conc.%
				Can Fungi	Juv Dens	% open	
1	55.7	11.6	0.0089	0.211	0.1366	0.0166	77
2	61.3	5.18	0.0749	0.0969	0.0446		63.9
3	56	9.29	0.0096	0.4875		0.0083	75.2
4	55.2	10.06	0.0065		0.2681	0.0101	72.6
5	62.5	0.85	0.3570	0.3498			23.8
6	60.9	2.42	0.1199		0.1211		47.4
7	54.5	8.82	0.0030			0.007	72.6

Model 7 was the best fit (lowest AIC with high concordance), taking the form  $p(\text{juvenile colonized}) = \text{expy} / (1 + \text{expy})$  where:  $y = -2.8624 + 0.2729(\% \text{ Canopy Openness})$ .

Fit of this model against the observed data are shown in Fig. E1.

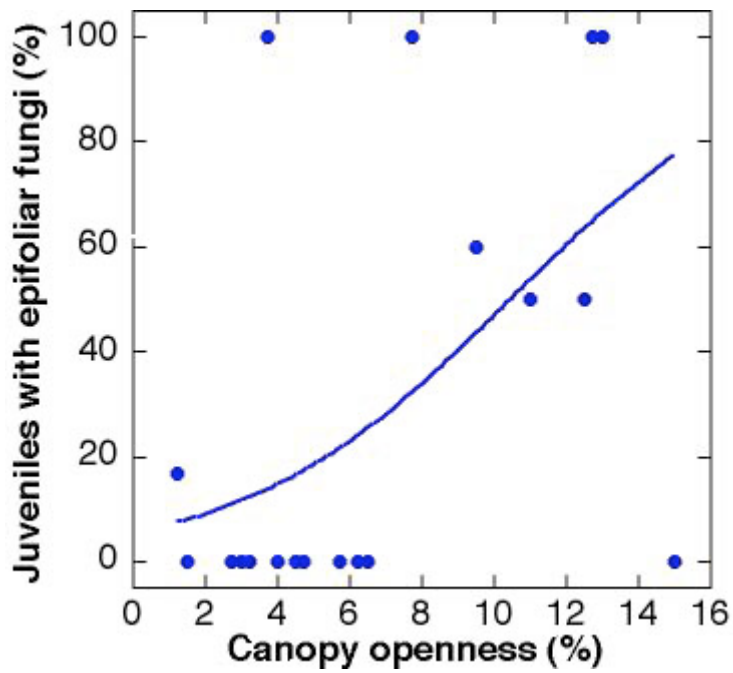


FIG. E1. Increased canopy openness increases the likelihood of epifoliar fungi on understory juveniles of *Cleistanthus myrianthus*. Each point is the percentage of juveniles within 3 m of an adult that were colonized by epifoliar fungi. The solid line indicates the probability that a juvenile would be colonized based on best-fit logistic regression ( $\text{logit}(\text{probability juvenile colonized}) = -2.8624 + 0.2729 (\text{canopy openness})$ ).

---

[\[Back to E088-035\]](#)