Incidence of Extrafloral Nectaries and Their Relationship with Growth and Survival of Lowland Tropical Rain Forest Trees

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ABSTRACT

Mutualistic relationships between organisms have long captivated biologists, and extrafloral nectaries, or nectar-producing glands, found on many plants are a good example. The nectar produced from these glands provides food for ants, which may defend the plant from potential herbivores in turn. However, relatively little is known about their impact on the long-term growth and survival of plants that produce them. To better understand the ecological significance of extrafloral nectaries, we examined their incidence on lowland tropical rain forest trees in Yasuní National Park in Amazonian Ecuador, and collated data from two other tropical lowland forest sites (Barro Colorado Island, Panamá and Pasoh Forest Reserve, Malaysia). At Yasuní, extrafloral nectaries were found on 137 of 1123 species censused (12.2%), widely distributed among different angiosperm families. This rate of incidence is high but consistent with other tropical locations. Furthermore, this study adds 18 new genera and two new families (Urticaceae and Caricaceae) to the list of taxa exhibiting extrafloral nectaries. Using demographic data from long-term forest dynamics plots at each site, we compared the growth and mortality rates of species with extrafloral nectaries to those without. After controlling for phylogeny, no general relationship between extrafloral nectary presence and demographic rates could be detected, suggesting little demographic signal from any community-wide ecological effects.

Abstract in Spanish is available with online material.

Key words: Barro Colorado Island; Ecuador; extrafloral nectaries; growth rate; Malaysia; mortality rate; Panama; Pasoh, tropical forest; Yasuní.

TROPICAL FORESTS REPRESENT A FASCINATING YET INCREDIBLY COMPLEX WEB OF INTERACTIONS, the ecology of which, in many cases, is still largely enigmatic, and the mechanisms that generate and maintain the remarkable diversity of plants and animals found within them remain a fundamental question in biology (Palmer 1994, Hubbell 2001, Wright 2002). While one suite of mechanisms is purely stochastic in nature (*e.g.,* Hubbell 2001) many other mechanisms depend on niche differences between species to permit coexistence (Chesson 2000, Silvertown 2004). Niche differences are driven primarily in response to selection pressures, which in tropical forests include competition with neighbors for (often very low levels of) light, nutrients, and water (Chapin *et al.* 1986, Denslow *et al.* 1987, Chazdon & Pearcy 1991), as well as intense predation pressure from pests, pathogens, and herbivores (Barone 2000, Novotny *et al.* 2010).

Herbivory represents a particularly selective force, as up to 20 percent of plant net primary production may be consumed each year (Agrawal 2011). In response, tropical rain forest trees

have developed a myriad of defense mechanisms, from physical (*e.g.,* spines, hairs; Hanley *et al.* 2007) to chemical (*e.g.,* low nutrition, toxic compounds; Feeny 1976, Levin & York 1978, Coley & Barone 1996). Further, many plants have evolved mutualistic relationships with animals in an effort to deter herbivores. A common mutualism is with ants, and such ant–plant relationships offer a considerable measure of defense from herbivory and can have a positive impact on plant performance (Beattie 1985, Heil & McKey 2003).

One such example of ant–plant mutualisms are extrafloral nectaries (EFNs), which are nectar-producing glands found outside of a plant's flower, typically at the base of the leaf or on the petiole, although their location can vary considerably on the plant. EFNs vary in morphology, ranging from raised bowls or bulbs to very small hairs and tissues (Elias 1983). The nectar produced by these glands serve as a food source, primarily for ants, which are believed to provide protection to the plant in return, by way of aggression toward intruding organisms including herbivores (Bentley 1977a, Keeler 1977, 1989, Koptur 1992, Rosumek *et al.* 2009). This form of ant protection can result in reduced damage to both vegetative and reproductive parts, suggesting improved plant performance and fitness (Koptur 1992, Oliveira

1997). They are also believed to indirectly facilitate increased speciation rates among clades that evolved EFNs by enabling another axis of plant–animal interaction (Weber & Agrawal 2014). However, relatively little is known about the effect of EFNs on the long-term performance of individual plants, or the overall ecological impact at the population or community level.

Previous intensive surveys have determined the incidence of EFNs on Barro Colorado Island, Panamá (Schupp & Feener 1991) and in the Pasoh Forest Reserve, Malaysia (Fiala & Linsenmair 1995). These studies provided an excellent picture of the distribution of EFNs at these sites, and until recently were the best data available on the phylogenetic distribution of EFNs. However, new work has drawn together all available data on EFN incidence currently known, to examine the phylogenetic distribution of EFNs throughout the plant phylogeny (Weber & Keeler 2013). This study found 1.0–1.8 percent of plant species had EFNs, distributed in 108 families, although the authors suggest that the unknown incidence of EFNs may be as great as the their currently known incidence (Weber & Keeler 2013), requiring further in-depth studies of EFN incidence within and between plant communities.

In this study, we expand upon our prior understanding by undertaking an intensive survey of EFN incidence of tree species in an old growth Neotropical aseasonal lowland rain forest, an environment that has not yet been studied for EFNs. We analyzed the long-term demographic rates of tree species with and without EFNs to elucidate the ecological significance of this defensive strategy. In a large permanent forest plot in Yasuní National Park, Ecuador, we examined 928 species of tree for the presence or absence of EFNs. We used published census data to compare abundance, and growth and mortality rates of trees with and without EFNs. Finally, we also used the results of published surveys from BCI and Pasoh (Condit *et al.* 2006) to examine how plant performance is related to EFN incidence at these sites, such that a comparison of the phylogenetic distribution and demographic rates related to EFN can be made at a wider scale. If mutualism with ants, and EFNs in particular, provide a benefit, we predict higher abundance and greater performance in species with EFNs. Specifically, we asked the following questions: (1) what is the incidence and phylogenetic distribution of extrafloral nectaries on trees in a Neotropical lowland rain forest? (2) Do tree species with extrafloral nectaries have greater abundance, lower mortality and higher growth rates than tree species without? (3) Are these patterns consistent across different biogeographic histories and forest types?

METHODS

Study sites.—We carried out fieldwork in Yasuní National Park, Ecuador, and used published data from Barro Colorado Island, Panamá (Schupp & Feener 1991) and Pasoh Forest Reserve, Malaysia (Fiala & Linsenmair 1995) on the incidence of EFN. Yasuní National Park and adjacent Huaorani territory comprise 1,600,000 ha of largely pristine tropical lowland aseasonal rain forest in eastern Ecuador (Finer *et al.* 2009, Bass *et al.* 2010). Yasuní Scientific Research Station, established and maintained by

the Pontificia Universidad Católica del Ecuador, is located in the northwestern corner of the park, in terra firme, mature forest bordering the Tiputini River. The research station maintains a 25-ha Forest Dynamics Plot (FDP, 041′ S, 7624′ W), which lies along two smaller ridges dominated by red clays and separated by a valley characterized by brown or gray alluvium (Valencia *et al.* 2004). The plot is extremely biologically diverse, with a described tree species count of 1159 (Valencia *et al.* 2004). The climate at Yasuní is aseasonal, with an average annual rainfall of 2826 mm, with no month receiving <100 mm of rainfall (Valencia *et al.* 2004).

Pasoh Forest Reserve, Malaysia is a 11,000 ha reserve situated in peninsular Malaysia. The 50-ha Forest Dynamics Plot situated within the reserve (258′ N, 10218′ E) was established in 1986 and is maintained by the Forest Research Institute Malaysia. The forest consists primarily of lowland mixed dipterocarp forest and is surrounded by roughly 1000 ha of previously logged forest. The FDP at Pasoh has a tree diversity of around 814 species. The climate at Pasoh is seasonal, with dips in precipitation in January–February and June–July, and an average annual rainfall of 1571 mm (Manokaran *et al.* 2004).

Barro Colorado Island (BCI), Panamá is a 1560 ha island located in Gatún Lake, formed when the Panamá Canal was developed. The 50-ha Forest Dynamics Plot was established in 1980 and is maintained by the Smithsonian Tropical Research Institute (STRI). The FDP is located near the center of BCI (99′ S, 7950′ W) and consists primarily of lowland moist tropical forest. There is a relatively high diversity of trees in the FDP, with 321 different species of tree recorded. The climate at BCI is seasonal, with a dry season lasting roughly from December to April or May and an average annual rainfall of 2551 mm (Leigh *et al.* 2004).

Field surveys.—We undertook a survey for incidence of extrafloral nectaries on woody species at Yasuní in June–August 2012. Species were censused in three ways. In the field, we searched along trails within and around the FDP and found 787 species. A further 141 rare species were found by searching for specific individuals within the FDP. In this way, we examined a total of 928 species in the field (80% of the total 1159 species in the FDP). For each species, we checked EFN incidence for one to five saplings or small trees. The remaining 231 species that we could not find in the field were checked from both dried specimens in the field station herbarium and with Pennington *et al.* (2004). The herbarium was effective for those plants with obvious nectary structures (*e.g.,* Fabaceae), although dried structures are much more difficult to identify than living structures.

Data on the incidence of extrafloral nectaries for tree species from the other two sites were obtained from Schupp and Feener (1991, BCI) and Fiala and Linsenmair (1995, Pasoh). We augmented these data with additional information from Keeler (2013), Croat (1978), Garwood and Tebbs (2009), and Soepadmo *et al.* (1995–2006).

Demographic data.—At all three sites, identical methodology was followed to establish large forest dynamics plots. All plots

were professionally surveyed, and within them every free-standing woody stem >1 cm diameter at breast height (dbh, 1.3 m) are mapped, marked, measured, and identified every 5 yr (Condit 1998). To date, three censuses have been carried out at Yasuní, seven at BCI and three at Pasoh. All demographic data can be found at the Center for Tropical Forest Science website (http://www.ctfs.si.edu).

From these census data, demographic rates have been calculated for each species (Condit *et al.* 2006). Annual mortality (survival from one census to the next) and growth rates (diameter increment) were determined using Bayesian hierarchical models. Abundance and demographic rates were calculated for each species for individuals in two size classes: 1–10 cm dbh and >10 cm dbh. Size classes were maintained for analysis to separate demographic trends between young/small trees and shrubs, and larger, as nectar production tends to be more active for earlier growth (Bentley 1977a). For consistency, census years leading up to or closest to the year 2000 were used. For each species, we also assigned growth form defined by the maximum height they usually attain: shrubs (<5 m), treelets (≥5 and <10 m), mid-canopy trees (≥10 and <20 m), and tall-canopy trees (≥20 m), following Valencia *et al.* (2004). Finally, the higher level taxonomy for each site was updated to reflect the Angiosperm Phylogeny Group III (APG III) system (Bremer *et al.* 2009).

PHYLOGENY.—For each plot, we constructed a community phylogeny using Phylomatic 3 (Webb & Donoghue 2005), a software utility that constructs a hypothesized phylogeny that includes the angiosperm species (which may be members of a given habitat or community) provided by the user. The relationships assigned to the listed species are based on the dynamic angiosperm phylogeny available online (Stevens 2001 onwards). Specifically, we used the maximally resolved seed plant tree designated 'R20120829', including only taxa for which we had information on EFN incidence. Branch lengths were assigned using the 'bladj' function in Phylocom 4.2 (Webb *et al.* 2001–2009) based on the ages described in Wikstrom *et al.* (2001). We repeated the two demographic analyses described above using a phylogenetic generalized least squares (pgls) approach, to account for the non-independence of taxa with a shared evolutionary history, using the 'caper 0.5.2' package (Orme *et al.* 2013; Queenborough *et al.* 2009).

DATA ANALYSIS.—For both size classes, we tested for differences in *ln*-transformed tree abundance, using ANOVA. Then we tested for differences in *ln*-transformed mortality rate and *ln*-transformed diameter relative growth rate as a function of EFN presence, using ANOVA. Finally, to examine how EFN presence related to the well-established growth-mortality trade-off in tropical trees (Swaine & Whitmore 1988, Welden *et al.* 1991, Brokaw & Busing 2000), we included diameter relative growth rate as a covariate in a model of mortality rate as a function of EFN presence for trees 1–10 cm dbh for each site.

We examined the distribution of EFNs on the community phylogenies, using the 'ape' (Paradis *et al.* 2004) and 'caper' (Orme *et al.* 2012) packages for R. We used the function 'phylo.d

()' to estimate the phylogenetic signal, D, of the binary trait (EFN) on each phlyogeny (Fritz & Purvis 2010). This function runs two simulations. First, trait values are randomly shuffled relative to the tips of the phylogeny, and a variable with random association will have $D \approx 1$. Second, a continuous trait is evolved along the phylogeny under a Brownian process and then converted to a binary trait using a threshold that reproduces the relative prevalence of the observed trait. Here, a variable following a Brownian model will have $D \approx 0$. Thus, values of D smaller than 0 are phylogenetically more conserved than under a Brownian model and values of $D > 1$ are phylogenetically overdispersed. All data analysis was completed in the statistics package R v. 3.2.0 R Foundation for Statistical Computing, Vienna, Austria.

RESULTS

We surveyed shrub and tree species at three tropical forest sites for extrafloral nectaries. At Yasuní, we censused 928 species of 1159 species on the FDP. At BCI, Schupp and Feener (1991) surveyed 173 species, though only 127 of these are present on the FDP (of 321 total). Using additional references, we added another 45 species with EFNs. At Pasoh, Fiala and Linsenmair (1995) surveyed 729 of 814 species, and we added a further one from Keeler (2013), and 16 from Soepadmo *et al.* (1995–2006). Thus, we have a good sample of the species at each site, and most of the unsurveyed species are rare. The species list with EFN presence from our survey at Yasuni can be found in Table S1.

TAXONOMIC DISTRIBUTION.—At Yasuní, we found 137 species with extrafloral nectaries (12.2% of the total 1159 species, Fig. S1). These were distributed among 54 genera and 23 families. The majority (66) of the species with EFNs were in the family Fabaceae (Fig. S2), largely thanks to the diversity of *Inga* (44 species) at Yasuní, all of which have EFNs. Of all species with EFN, 68 percent were in the orders Fabales or Malpighiales. In addition, we documented 18 new genera and two new families (Caricaceae and Urticaceae) with EFNs, expanding the global list of taxa with EFNs (Keeler 2013).

At Pasoh, 85 (11.4%) of 814 species were found to have EFNs (Fiala & Linsenmair 1995, plus additional data, Fig. S1). They were distributed among 42 genera and 16 families (Fig. S2). Unlike Yasuní and BCI, Pasoh exhibited a more even distribution of EFN-bearing trees across different taxa. Euphorbiaceae, rather than Fabaceae, contained the most species with EFNs (23 species). Of all species with EFNs, 44 percent were in the order Malpighiales, while the next most important order was Malvales (16.5%).

At BCI, 67 (22.9%) of 293 species of tree had EFNs (Schupp & Feener 1991, plus additions, Fig. S1). These species were distributed among 48 genera and 25 families (Fig. S2). Within the Fabaceae, 19 species had EFNs, also due largely to the diversity of *Inga* (15 species). Similar to Yasuní, 55 percent of

all species with EFNs were found within either the Fabales or Malpighiales orders.

Across all study sites, Yasuní and Pasoh were most similar in their proportion of species with EFNs (12.2% in Yasuní, 11.4% in Pasoh). BCI exhibited an incidence of species with EFNs two times greater than Yasuní and Pasoh (22.9%, Fig. S1), and at all taxonomic levels, BCI had greater proportions of plants with EFNs than both Yasuní and Pasoh. However, BCI exhibited <50 percent incidence of EFNs at the individual level compared with the other study sites.

ABUNDANCE, AND GROWTH AND MORTALITY RATES.—Species varied widely in their abundances, growth and mortality rates (Figs. 1 and 2). Species at BCI were on average two times more abundant than species from Yasuní and Pasoh, reflecting the lower species richness found at BCI. Demographic rates also tended to be higher at BCI.

In accordance with our prediction, species with EFN were significantly more abundant than species without EFNs at Yasuní (trees >10 cm dbh only, Table 1A iii, detailed test statistics can be found in Table 1) and Pasoh (both size classes, Table 1A ix), but this was not the case at BCI where trees with EFN were significantly less abundant than trees without EFN (in the 1–10 cm dbh size class only, Table 1A vi, Fig. 1A–C). At Yasuní, species abundances for trees with EFNs were on average 12.5 percent greater than those without. At Pasoh, these differences were even greater, where those species with EFNs were 66 percent more abundant than those without. In contrast, at BCI trees in the small size class without EFNs were considerably more abundant.

Significantly greater growth rates were found in trees with EFNs in each plot, although this differed with size class between sites (Fig. 1D–F). At Yasuní, trees with EFNs in both size classes had higher growth rates (Table 1A i): trees 1–10 cm dbh grew on average 0.23 mm per mm per year more than species without EFNs, and trees >10 cm dbh grew on average 0.22 mm per mm per year more. At Pasoh, trees 1–10 cm dbh with EFN grew on average 0.08 mm per mm per year more than trees without EFN (Table 1A vii). At BCI trees >10 cm dbh with EFN grew 0.31 mm per mm per year more on average (Table 1A iv).

Significantly higher mortality rates were found for species with EFN in the both size classes at all three sites (Table 1A ii, v, viii), aside from BCI where higher mortality rates were found only in trees >10 cm dbh, Fig. 1G–I).

Furthermore, when we accounted for the relationship between mortality and growth, species with EFN at Pasoh had significantly higher mortality rates than species without (Fig. 1). There was no significant difference between species with EFN and without EFN at Yasuní and BCI.

GROWTH FORMS.—The majority of species and individuals with EFN at each site were in the forest canopy (mid- or tall-canopy trees, Fig. 3). Very few shrubs and understorey trees had EFN.

PHYLOGENY.—The phylogenetic distribution of EFN varied at each site (Fig. S3). Values of D were significantly different from

one at all sites, suggesting that the evolution of EFN did not follow a random phylogenetic structure. Instead, while values of D at all sites were <0, they were not significantly so, implying a model of Brownian evolution.

When we modeled demographic rates as a function of EFN presence accounting for the phylogenetic relationships among taxa, we found fewer differences between species with and without EFN (Fig. 1), although this varied by rate. Differences in abundance were maintained at Pasoh (Table 1B ix) and BCI (1–10 cm, Table 1B vi), but all significant differences in growth rates were not significant under the phylogenetic model (Fig. 1 D–F), and most differences disappeared for mortality (Fig. 1G–I). Moreover, in all cases, even when a significant difference was found, the absolute difference decreased, with coefficients for taxa with and without EFN estimated as much more similar than for the non-phylogenetic analyses. Similarly, when we modeled mortality as a function of growth rates and EFN incidence, the phylogenetic analysis dampened overall differences in demographic rates (Fig. 2). Furthermore, when we excluded dominant taxa with EFN from these models (Fabaceae from Yasuni and BCI, Euphorbiaceae from Pasoh, see Figs. S2 and 4), most significant differences in demographic rates between taxa with and without EFN also disappeared (Fig. 4), suggesting that the differences found in the original non-phylogenetically corrected analyses were driven by these two families.

DISCUSSION

From an intensive field survey of 928 tree and shrub species in an Amazonian lowland tropical rain forest and outside references, we documented 137 species with extrafloral nectaries, 104 of which had not previously been recorded as possessing EFNs. Comparing Yasuní with two other intensive survey sites, we found that the distribution of EFNs across taxa was consistent between Yasuní and Pasoh, and broader at BCI. The reverse was found to be true in terms of total EFN presence on individuals at each plot. Species with EFNs appeared to be more successful ecologically at Yasuní and Pasoh, having higher abundances than species without EFNs, while at BCI the opposite was true. However, after controlling for phylogenetic relatedness, few significant correlations between EFN presence and plant performance were detected. Tree species with EFNs showed higher growth and mortality rates compared to those without EFNs at all three sites, but this was likely driven by clustering of EFNs in specific clades such as Fabaceae and Euphorbiaceae.

TAXONOMIC DISTRIBUTION AT YASUNÍ.—This study adds 18 new genera and two new families (Caricaceae and Urticaceae; Table S1) to the list of taxa exhibiting EFNs. This increases the global number of families with EFNs to 110, 19 of which are found at Yasuní. The family with the most number of EFNs at Yasuní was Fabaceae, which is also true globally. However, the family Euphorbiaceae had the second highest incidence of EFNs at Yasuní, which stands in contrast to global patterns which show
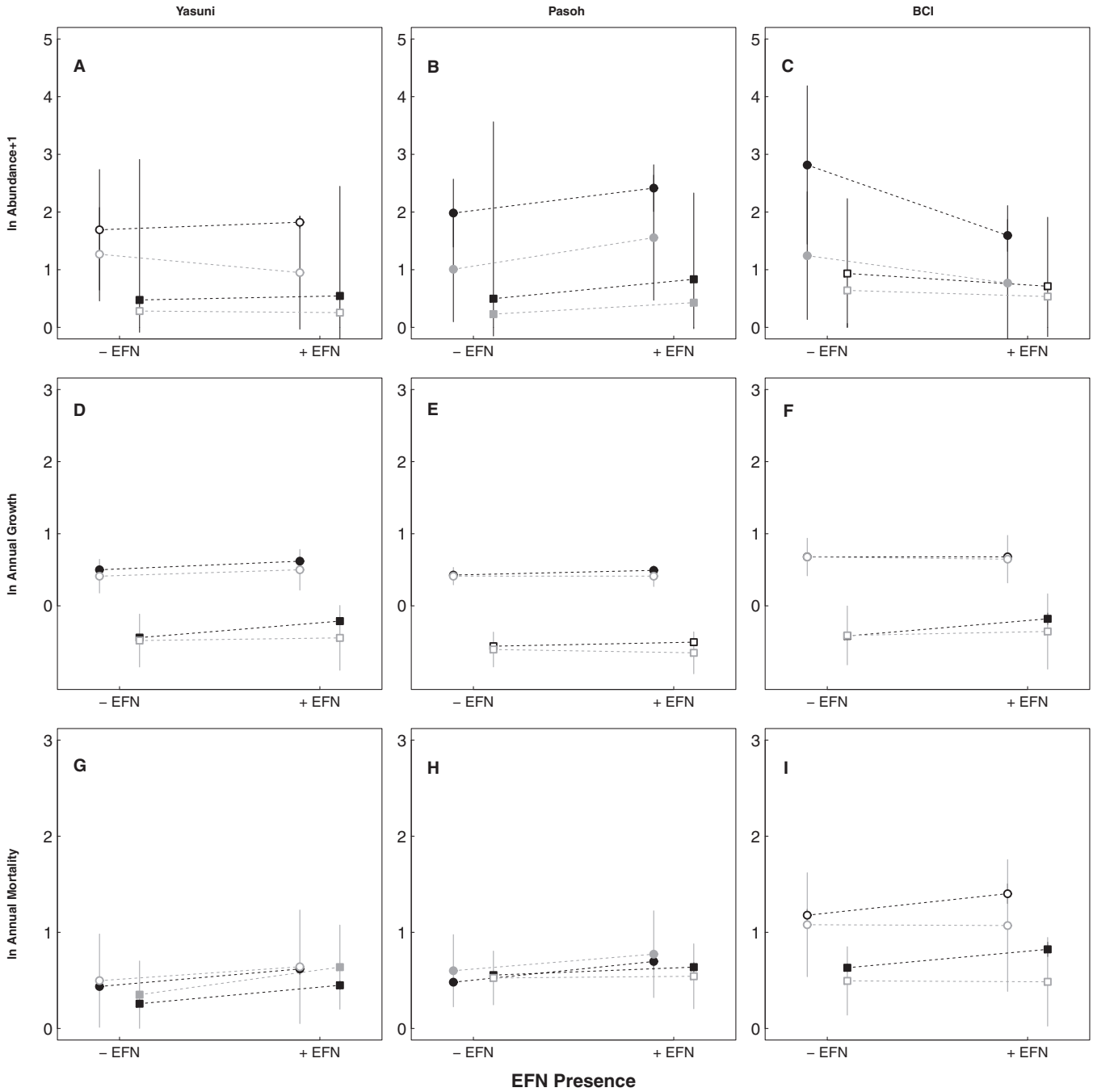
FIGURE 1. Abundance (A, B, C), and growth (D, E, F) and mortality (G, H, I) rates as a function of EFN presence (+) and absence (−) in shrub and tree species in three tropical forest sites. Points are mean values split into two size classes: trees 1–10 cm dbh (circles), and trees >10 cm dbh (squares), each size class connected by dashed lines. Black lines and symbols indicate a 'tips' analysis where all species were treated as independent; gray lines and symbols indicate a phylogenetic generalized least squares model, where the phylogenetic signal of extrafloral nectaries and demographic rates was accounted for. Filled symbols indicate statistical difference species with nectaries (+EFN) from those without (−EFN) (ANOVA, $P < 0.05$). Error bars show SE (abundance) or 95% CIs (growth and mortality).

Passifloraceae and Malvaceae as second and third, respectively. Only one species of Malvaceae had nectaries at Yasuní, while there were none from Passifloraceae (although we did not survey any vines and lianas, the predominant growth form of Passifloraceae). The presence of EFNs at Yasuní was much greater than the currently known worldwide incidence (12.2% at Yasuní, com-

pared to *ca* 1.5% of all flowering plant species worldwide, Weber & Keeler 2013).

TAXONOMIC DISTRIBUTION BETWEEN SITES.—All three locations exhibited fairly equivalent distribution of EFNs across taxa, with BCI representing the greatest breadth of distribution, and the
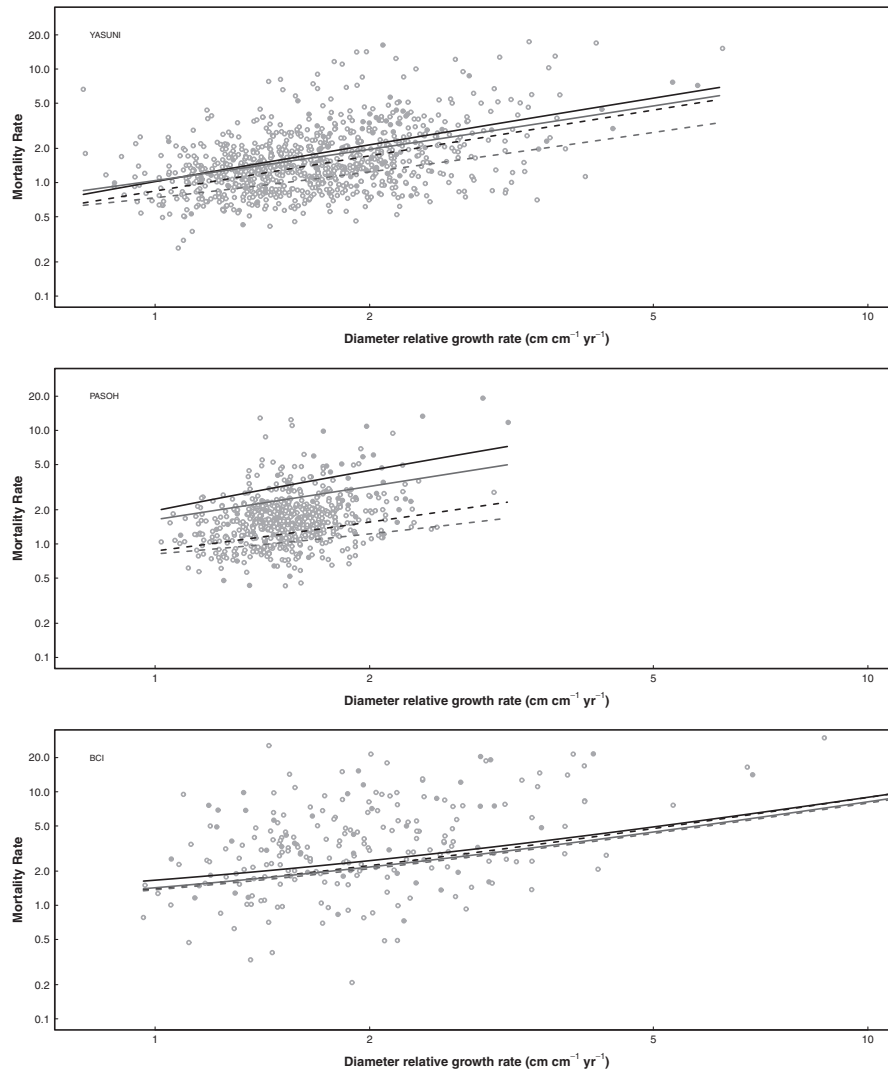
FIGURE 2. The trade-off between mortality and growth in trees 1–10 cm dbh at three tropical forest sites, testing for differences between species with (filled point, solid line) and without (open points, dotted line) extrafloral nectaries. Black lines and symbols indicate a 'tips' analysis where all species were treated as independent; gray lines and symbols indicate a phylogenetic generalized least squares model, where the phylogenetic signal of extrafloral nectaries and demographic rates was accounted for. Each point represents the mean demographic rates of a species. Lines are fitted regression lines from ANCOVAs with different intercepts and slopes. BCI lines for species without EFNs are overlapped by the species line with EFNs (solid line).

least negative phylogenetic signal. Oddly, BCI also exhibited the lowest total number of individuals with EFNs, despite the wide taxonomic distribution and greater number of species relative to Pasoh and Yasuní. Pasoh, which overall had the smallest phylogenetic distribution and species count of those trees with EFNs, had the greatest number of individuals with nectaries. This result may in part be because many dipterocarps, the dominant family in SE Asian forests, have EFNs.

In Yasuní, BCI and Pasoh, the orders Fabales and Malpighiales were well represented by species with EFNs, with at least 10 species with EFN being found in each order. Unlike in BCI and Yasuní, the orders Ericales and Malvales were also found to have at least ten species with EFNs in Pasoh. As such, despite an overall smaller distribution of EFNs across orders in Pasoh (30%

in Pasoh, as opposed to 43% at Yasuní and 50% at BCI), more families were well represented by species with EFNs. BCI and Yasuní, then, have a thinner distribution of EFNs across orders. This is generally the case for families as well, as those orders with many EFN-bearing species in Pasoh are this way due to particularly well-represented families (Dipterocarpaceae, Ebenaceae, Euphorbiaceae, and Fabaceae). This, in large part, reflects the different floristic composition of Paleo versus Neotropical forests (Gentry 1993).

GEOGRAPHIC DISTRIBUTION.—An increase in EFN presence as latitude decreases has been noted previously (Pemberton 1998), but it is also informative to examine how EFN distribution changes across different habitat types at similar latitudes. Yasuní and

TABLE 1. *Summary results of models of log diameter growth rate, population mortality rate, and population abundance as a function of extrafloral nectary (EFN) incidence (±) for populations of tropical forest trees in three large forest dynamics plots, for both phylogenetically corrected and uncorrected data and two size classes of tree (1–10 cm dbh and >10 cm dbh).*

| | | 1–10 cm dbh | | | | | >10 cm dbh | | | | |
| | | −EFN | +EFN | | | | −EFN | +EFN | | | |
		mean ± SE	mean ± SE	df	t	P	mean ± SE	mean ± SE	df	t	P
A. No Phylogenetic Information											
Yasuni	i. Growth	0.50 ± 0.01	0.62 ± 0.04	914	3.95	<0.001	−0.44 ± 0.02	−0.22 ± 0.06	726	5.27	<0.001
	ii. Mortality	0.44 ± 0.02	0.62 ± 0.08	989	2.91	<0.01	0.26 ± 0.02	0.45 ± 0.06	771	4.58	<0.001
	iii. Abundance	1.07 ± 0.03	1.01 ± 0.12	1080	−0.67	0.5	0.29 ± 0.01	0.38 ± 0.06	1080	2.22	0.026
BCI	iv. Growth	0.68 ± 0.03	0.68 ± 0.09	236	0.01	0.99	−0.42 ± 0.04	−0.18 ± 0.12	203	2.81	<0.01
	v. Mortality	1.18 ± 0.06	1.40 ± 0.19	263	1.76	0.08	0.63 ± 0.04	0.82 ± 0.12	212	2.33	0.021
	vi. Abundance	1.36 + 0.09	0.87 ± 0.28	291	−2.65	<0.01	0.52 ± 0.05	0.44 ± 0.15	291	−0.86	0.39
Pasoh	vii. Growth	0.43 ± 0.01	0.49 ± 0.03	655	3.47	<0.001	−0.56 ± 0.01	−0.50 ± 0.05	565	1.48	0.14
	viii. Mortality	0.48 ± 0.02	0.70 ± 0.08	667	3.43	<0.001	0.56 ± 0.01	0.64 ± 0.05	576	2.02	0.044
	ix. Abundance	1.26 ± 0.04	1.68 ± 0.18	740	3.36	<0.001	0.37 ± 0.02	0.60 ± 0.07	740	4.19	<0.001
B. Phylogenetic Correction											
Yasuni	i. Growth	0.41 ± 0.23	0.50 ± 0.28	767	1.83	0.067	−0.48 ± 0.37	−0.44 ± 0.45	612	0.45	0.66
	ii. Mortality	0.50 ± 0.49	0.64 ± 0.59	816	1.37	0.17	0.35 ± 0.35	0.64 ± 0.44	652	3.29	<0.01
	iii. Abundance	1.26 ± 0.81	0.95 ± 0.98	863	−1.85	0.065	0.28 ± 0.37	0.26 ± 0.45	863	−0.35	0.73
BCI	iv. Growth	0.68 + 0.26	0.65 ± 0.33	235	−0.43	0.67	−0.41 ± 0.41	−0.36 ± 0.52	203	0.48	0.63
	v. Mortality	1.08 ± 0.54	1.07 ± 0.69	262	−0.06	0.95	0.50 ± 0.36	0.49 ± 0.46	212	−0.09	0.93
	vi. Abundance	1.24 ± 1.11	0.77 ± 1.34	289	−2.02	0.044	0.64 ± 0.57	0.53 ± 0.69	289	−0.88	0.38
Pasoh	vii. Growth	0.41 ± 0.12	0.41 ± 0.14	655	−0.01	0.99	−0.61 ± 0.24	−0.65 ± 0.29	565	−0.93	0.35
	viii. Mortality	0.60 ± 0.37	0.77 ± 0.45	667	2.27	0.024	0.53 ± 0.28	0.54 ± 0.34	576	0.3	0.77
	ix. Abundance	1.01 ± 0.91	1.56 ± 1.08	740	3.14	<0.01	0.23 ± 0.38	0.43 ± 0.45	740	2.73	<0.01
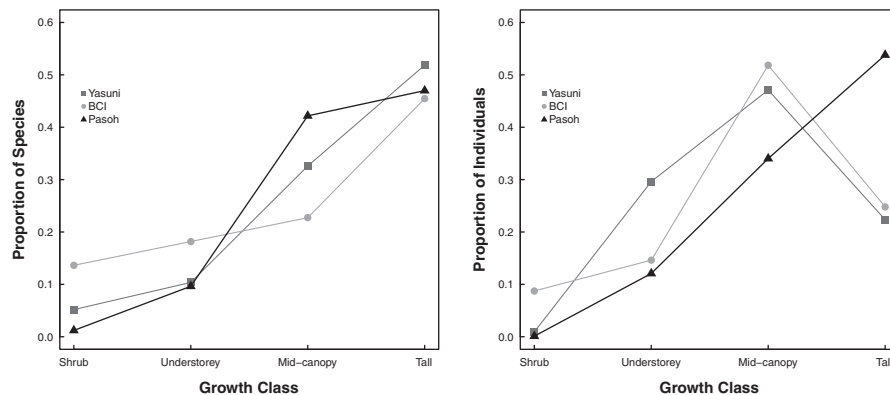
FIGURE 3. Proportion of species and total individuals with EFNs across varying growth classes at three tropical forest sites. Each proportion is out of the total number of species or individuals bearing EFNs.

Pasoh, which are two lowland tropical rain forests at comparable latitudes, are very similar in their incidence of EFNs, suggesting little difference in distribution patterns between the Neotropics and Paleotropics, as represented by these two sites. The slightly higher incidence in the Neotropics may be attributed to the greater diversity of Fabaceae found there (Gentry 1993).

Within the Neotropics, the Brazilian Cerrado savanna vegetation has also been surveyed for the presence of EFNs. An incidence of about 17 percent of woody plants with EFNs was found in the Cerrados, which are considerably drier and more open than rain forest (Oliveira & Leitõ-Filho 1987). Perhaps, the greater presence of EFNs found in the Cerrado indicates that ant–plant interactions are stronger in this type of habitat, or that
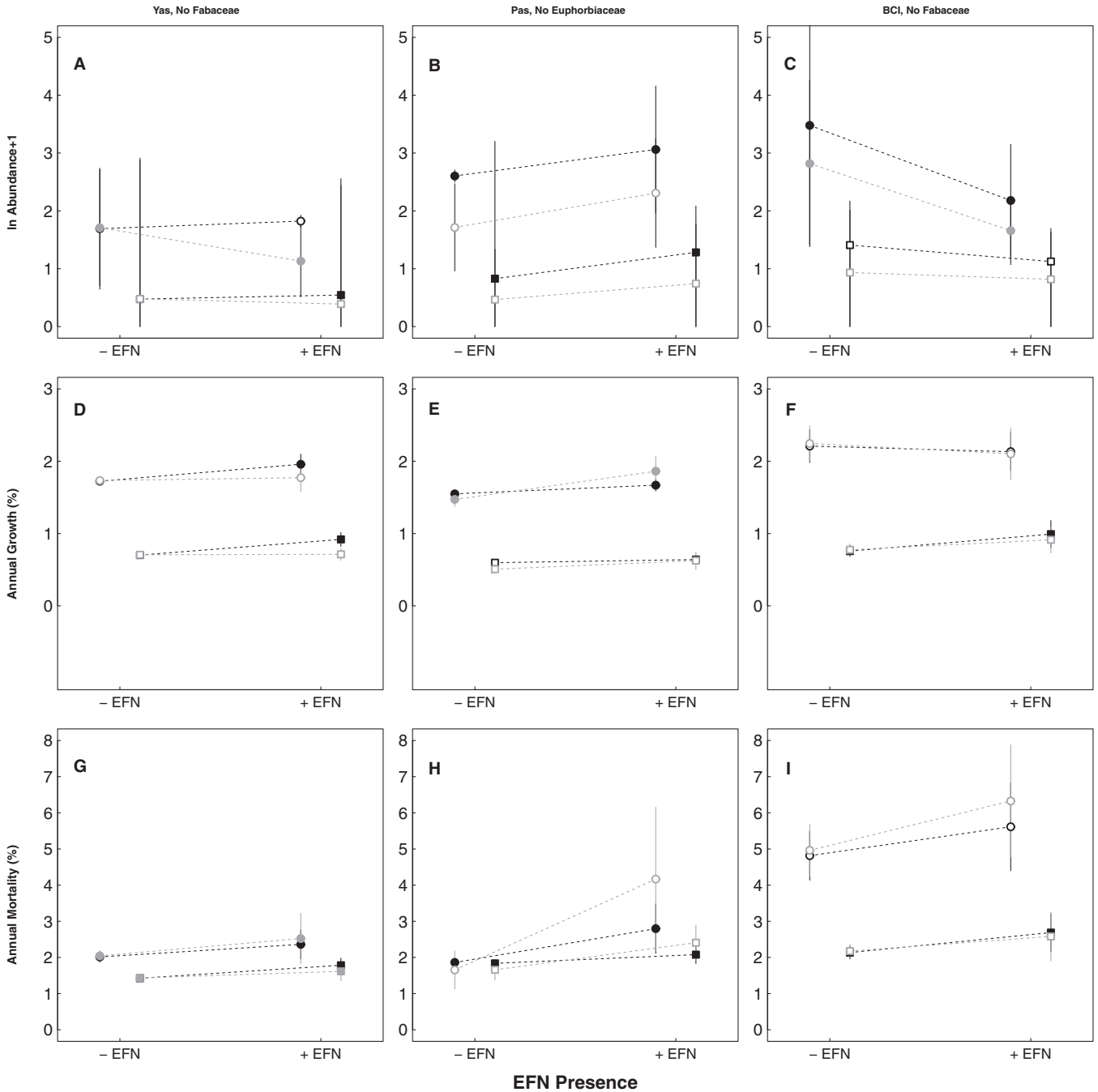
FIGURE 4. Abundance (A, B, C), and growth (D, E, F) and mortality (G, H, I) rates as a function of EFN presence (+) and absence (−) in shrub and tree species in three tropical forest sites. Points are mean values split into two size classes: trees 1–10 cm dbh (circles), and trees >10 cm dbh (squares), each size class connected by dashed lines. Black lines and symbols indicate a 'tips' analysis where all species were treated as independent; gray lines and symbols indicate the same analysis but with the family Fabaceae excluded for Yasuni and BCI, and Euphorbiaceae excluded for Pasoh. Filled symbols indicate statistical difference species with nectaries (+EFN) from those without ((−EFN) (ANOVA, $P < 0.05$).

the cost of producing EFNs is lower because of the higher light availability and less light-limitation of photosynthesis in this habitat (Graham *et al.* 2003). Further work in understanding the differences in ant diversity and presence between these two habitats

may better inform our understanding of this mutualism across geographically similar habitats.

GROWTH FORMS.—An increase in proportion of species with EFNs as growth form size increases may indicate that the pres-

ence of EFNs more effectively deters herbivory in taller canopy trees compared with shorter understory shrubs and trees. This interpretation assumes that EFN nectar production is consistent across tree life stages, which is unlikely—seedlings, saplings, and the young leaves of mature trees frequently have more active nectar production than old leaves. If nectar production is only relevant at early life-history stages such as seedlings, when size across growth forms is homogenous, then there should be no interaction between growth form and EFN presence. However, because EFNs were more prevalent in mid-canopy and tall trees, it is possible that their adaptive value persists at older life stages. This trend may also suggest that only species with access to high light availability have the resources available to produce nectar for ants. Better understanding the significance of EFNs on seedlings and saplings as well as nectar production patterns through leaf ontogeny may help explain this observation.

ABUNDANCE.—Differences in abundance largely dissipated in the Neotropical locations after analyses corrected for phylogeny, but persisted at Pasoh because of the high abundances of Dipterocarpaceae (both with and without EFN). The families Fabaceae, Euphorbiaceae, and Dipterocarpaceae are all dominant and speciose families in tropical lowland forests, with a wide variety of ecological strategies.

ECOLOGICAL SIGNIFICANCE OF EXTRAFLORAL NECTARIES.—We correlated the presence of EFNs with demographic rates, such as growth and mortality, in an attempt to detect a signal of the impact that EFNs have in tropical forest communities. Differences were found, with EFN-bearing species exhibiting higher growth and mortality rates, but these differences largely disappeared after accounting for phylogenetic relatedness, indicating that these patterns are driven primarily by a few EFN-bearing clades. This is certainly the case in Yasuni and BCI, where Fabaceae likely drives these differences, as some of its diverse EFN-bearing genera, *e.g., Inga*, are also known to exhibit fast growing, short-lived life-history strategies. Indeed, when Fabaceae was removed from the original analysis, differences in growth rates disappeared at Yasuni and BCI. Differences in mortality rates when removing Fabaceae did not track phylogenetically corrected results, however, and no single family could completely explain the differences. Further, changes in mortality differences following phylogenetic correction were relatively small, thus making it difficult to pinpoint the taxa primarily driving these differences. Regardless, Fabaceae is largely responsible for differences in growth at these sites, while affirming that a small increase in mortality rates at Yasuni persists in EFN-bearing species after accounting for phylogeny. Phylogenetically driven differences in demographic rates at Pasoh were primarily due to Euphorbiaceae, the family with the most EFN-bearing species, as both growth and mortality differences disappeared when removed from the 'tips' analysis.

Thus, while there appears to be little direct correlation between EFN incidence and the ecological performance of trees >1 cm dbh, EFNs are more common in dominant, speciose families such as Fabaceae, Euphorbiaceae, and Dipterocarpaceae, suggesting a long-term evolutionary effect. Recent work has shown that EFN presence tends to increase diversification rates within clades (Weber & Agrawal 2014), a trend supported in Yasuni and BCI, where Fabaceae contributed the greatest EFN-bearing and total species diversity at each site. However, EFNs are absent from the four most speciose families in Pasoh, but are nonetheless common in Euphorbiaceae and Dipterocarpaceae, both highly abundant families. There is evidence by association, then, that EFN presence may be related to the evolutionary success of these clades. Furthermore, this evolutionary success must have some basis in ecological success: Given that the structure of tropical tree communities is generally well-established by the time trees are large enough to be included in these plot censuses (1 cm dbh, Baldeck *et al.* 2013), important processes must occur during earlier life stages undocumented in this study, such as the effect of EFN on the performance of seedlings and saplings.

CONCLUSIONS

We determined the phylogenetic distribution of extrafloral nectaries in a comprehensive survey of tree species in an aseasonal lowland rain forest in Ecuador, and compared this to previous surveys in lowland semi-deciduous moist forest in Panamá and lowland seasonal rain forest in Malaysia. A significant correlation of EFN presence with tree performance was not robust to phylogenetic correction, suggesting that EFNs occur and have ecological effects within a limited array of taxa. These taxa are some of the most abundant and speciose families in tropical lowland forests, suggesting an effect of EFNs on speciation and diversification that may be based in increased fitness of seedlings rather than established trees. Thus, the significant role of extrafloral nectaries and plant defense mechanisms in general in determining forest structure and composition may be played out over longer time-scales than current demographic data can inform.

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SUPPORTING INFORMATION

Additional Supporting Information may be found with online material:

TABLE S1. *Survey results at Yasuni from all sources.*

FIGURE S1. Proportion of trees >1 cm dbh at three tropical forest sites with extrafloral nectaries, at the individual and increasing taxonomic levels.

FIGURE S2. Number of species per family at each of three tropical forest sites with and without extrafloral nectaries.

FIGURE S3. Phylogenetic distribution of extrafloral nectaries among species of the Yasuni Forest Dynamics Plot, Ecuador.

FIGURE S4. Phylogenetic distribution of extrafloral nectaries among species of the Barro Colorado Island Forest Dynamics Plot, Panama.

FIGURE S5. Phylogenetic distribution of extrafloral nectaries among species of the Pasoh Forest Dynamics Plot, Malaysia.

LITERATURE CITED

AGRAWAL, A. A. 2011. Current trends in the evolutionary ecology of plant defence. Funct. Ecol. 25: 420–432.

BALDECK, C. A., K. E. HARMS, J. B. YAVITT, R. JOHN, B. L. TURNER, R. VALENCIA, S. NAVARRETE, S. BUNYAVEJCHEWIN, S. KIRATIPRAYOON, A. YAACOB, M. N. N. SUPARDI, S. J. DAVIES, S. P. HUBBELL, G. B. CHUYONG, D. KENFACK, D. W. THOMAS, AND J. W. DALLING. 2013. Habitat filtering across tree life stages in tropical forest communities. Proc. R. Soc. B Biol. Sci. 280: 20130548.

BARONE, J. A. 2000. Comparison of herbivores and herbivory in the canopy and understory for two tropical tree species. Biotropica 32: 307–317.

BASS, M. S., M. FINER, C. N. JENKINS, H. KREFT, D. F. CISNEROS-HEREDIA, S. F. MCCRACKEN, N. C. A. PITMAN, P. H. ENGLISH, K. SWING, G. VILLA, A. DI FIORE, C. C. VOIGT, AND T. H. KUNZ. 2010. Global conservation significance of Ecuador's Yasuní National Park. PLoS ONE 5: e8767.

BEATTIE, A. J. 1985. The evolutionary ecology of ant-plant mutualisms. Cambridge University Press, Cambridge, U.K.

BENTLEY, B. L. 1977a. Extrafloral nectaries and protection by pugnacious bodyguards. Annu. Rev. Ecol. Syst. 8: 407–427.

BENTLEY, B. L. 1977b. The protective function of ants visiting the extrafloral nectaries of Bixa orellana (Bixaceae). J. Ecol. 65: 27–38.

BREMER, B., K. BREMER, M. CHASE, M. FAY, J. REVEAL, D. SOLTIS, P. SOLTIS, AND P. STEVENS. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. 161: 105–121.

BROKAW, N., AND R. T. BUSING. 2000. Niche versus chance and tree diversity in forest gaps. Trends Ecol. Evol. 15: 183–188.

CHAPIN, III, F. S., P. M. VITOUSEK, AND K. VAN CLEVE. 1986. The nature of nutrient limitation in plant communities. Am. Nat. 127: 48–58.

CHAZDON, R. L., AND R. W. PEARCY. 1991. The importance of sunflecks for forest understory plants. Bioscience 41: 760–766.

CHESSON, P. 2000. Mechanisms of maintenance of species diversity. Annu. Rev. Ecol. Syst. 31: 343–366.

COLEY, P. D., AND J. A. BARONE. 1996. Herbivory and plant defenses in tropical forests. Annu. Rev. Ecol. Syst. 27: 305–335.

CONDIT, R. 1998. Tropical Forest Census Plots: Methods and results from Barro Colorado Island, Panama and a comparison with other plots. Springer Verlag, Berlin.

CONDIT, R., P. ASHTON, S. BUNYAVEJCHEWIN, H. S. DATTARAJA, S. DAVIES, S. ESUFALI, C. EWANGO, R. FOSTER, I. A. U. N. GUNATILLEKE, C. V. S. GUNATILLEKE, P. HALL, K. E. HARMS, T. HART, C. HERNANDEZ, S. HUBBELL, A. ITOH, S. KIRATIPRAYOON, J. LAFRANKIE, S. LOO DE LAO, J. MAKANA, S. NOOR, A. R. KASSIM, S. RUSSO, R. SUKUMAR, C. SAMPER, H. S. SURESH, S. TAN, S. THOMAS, R. VALENCIA, M. VALLEJO, G. VILLA, AND T. ZILLIO. 2006. The importance of demographic niches to tree diversity. Science 313: 98–101.

CROAT, T. B. 1978. Flora of Barro Colorado Island. Stanford University Press, Redwood City, California, USA.

DENSLOW, J. S., P. M. VITOUSEK, AND J. C. SCHULTZ. 1987. Bioassays of nutrient limitation in a tropical rain forest soil. Oecologia 74: 370–376.

ELIAS, T. S. 1983. Extrafloral nectaries: Their structure and distribution. In B. L. Bentley, and T. S. Elias (Eds.). The biology of nectaries, pp. 174–203. Columbia University Press, New York City, New York, USA.

FEENY, P. 1976. Plant apparency and chemical defense. In J. W. Wallace (Ed.). Biochemical interaction between plants and insects, pp. 1–40. Plenum Press, Berlin, Germany.

FIALA, B., AND K. E. LINSENMAIR. 1995. Distribution and abundance of plants with extrafloral nectaries in the woody flora of a lowland primary forest in Malaysia. Biodivers. Conserv. 4: 165–182.

FINER, M., V. VIJAY, F. PONCE, C. N. JENKINS, AND T. R. KAHN. 2009. Ecuador's Yasuní Biosphere Reserve: A brief modern history and conservation challenges. Environ. Res. Lett. 4: 034005.

FRITZ, S. A., AND A. PURVIS. 2010. Selectivity in mammalian extinction risk and threat types: A new measure of phylogenetic signal strength in binary traits. Conserv. Biol. 24: 1042–1051.

GARWOOD, N. C., AND M. C. TEBBS. 2009. Seedlings of Barro Colorado Island and the neotropics. Comstock Pub Assoc, Ithaca, New York, USA.

GENTRY, A. H. (Ed.). 1993. Four neotropical rainforests. Yale University Press, Binghamton, NY.

GRAHAM, E. A., S. S. MULKEY, K. KITAJIMA, N. G. PHILLIPS, AND S. J. WRIGHT. 2003. Cloud cover limits net CO_2 uptake and growth of a rainforest tree during tropical rainy seasons. Proc. Natl Acad. Sci. USA 100: 572–576.

HANLEY, M. E., B. B. LAMONT, M. M. FAIRBANKS, AND C. M. RAFFERTY. 2007. Plant structural traits and their role in anti-herbivore defence. Perspect. Plant Ecol. Evol. Syst. 8: 157–178.

HEIL, M., AND D. MCKEY. 2003. Protective ant-plant interactions as model systems in ecological and evolutionary research. Annu. Rev. Ecol. Evol. Syst. 34: 425–453.

HUBBELL, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, NJ.

KEELER, K. H. 1977. The extrafloral nectaries of Ipomoea carnea (Convolvulaceae). Am. J. Bot. 64: 1182–1188.

KEELER, K. H. 1989. Ant-plant interactions. In W. G. Abrahamson (Ed.). Plant-animal interactions, pp. 207–242. McGraw-Hill, New York City, New York, USA.

KEELER, K. H. 2013. World List of Angiosperm Species with Extrafloral Nectaries. Retrieved from http://biioscilabs.unl.edu/Emeriti/keeler/extrafloral/worldlistfamilies.htm

KOPTUR, S. 1992. Extrafloral nectary-mediated interactions between insects and plants. Insect Plant Interact. 4: 81–129.

Leigh, Jr, E. G., S. Loo de Lao, R. Condit, S. P. Hubbell, R. B. Foster, and R. Perez. 2004. Barro Colorado Island forest dynamics plot, Panama. In E. C. Losos, and E. G. Leigh (Eds.). Tropical forest diversity and dynamism: Findings from a large-scale plot network, pp. 451–463. University of Chicago Press, Chicago, Illinois, USA.

Levin, D. A., and B. M. York, Jr. 1978. The toxicity of plant alkaloids: An ecogeographic perspective. Biochem. Syst. Ecol. 6: 61–76.

Manokaran, N., Q. E. Seng, P. S. Ashton, J. V. LaFrankie, N. S. M. Noor, W. M. S. W. Ahmad, and T. Okuda. 2004. Pasoh forest dynamics plot, Peninsular Malaysia. In E. C. Losos, and E. G. Leigh (Eds.). Tropical forest diversity and dynamism: Findings from a large-scale plot network, pp. 585–598. University of Chicago Press, Chicago, Illinois, USA.

Novotny, V., S. E. Miller, L. Baje, S. Balagawi, Y. Basset, L. Cizek, K. J. Craft, F. Dem, R. A. Drew, J. Hulcr, J. Leps, O. T. Lewis, R. Pokon, A. J. Stewart, G. A. Samuelson, and G. D. Weiblen. 2010. Guild specific patterns of species richness and host specialization in plant-herbivore food webs from a tropical forest. J. Anim. Ecol. 79: 1193–1203.

Oliveira, P. S. 1997. The ecological function of extrafloral nectaries: Herbivore deterrence by visiting ants and reproductive output in Caryocar brasiliense (Caryocaraceae). Funct. Ecol. 11: 323–330.

Oliveira, P. S., and H. F. Leitõ-Filho. 1987. Extrafloral nectaries: Their taxonomic distribution and abundance in the woody flora of cerrado vegetation in Southeast Brazil. Biotropica 19: 140–148.

Orme, D., R. P. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2013. caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5.

Palmer, M. W. 1994. Variation in species richness: Towards a unification of hypotheses. Folia Geobot. Phytotaxon. 29: 511–530.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289–290.

Pemberton, R. W. 1998. The occurrence and abundance of plants with extrafloral nectaries, the basis for antiherbivore defensive mutualisms, along a latitudinal gradient in east Asia. J. Biogeogr. 25: 661–668.

Pennington, T. D., C. Reynel, and A. Daza. 2004. Illustrated guide to the trees of Peru. David Hunt, Sherborne, England.

Queenborough, S. A., S. J. Mazer, S. M. Vamosi, N. C. Garwood, R. Valencia, and R. P. Freckleton. 2009. Seed mass, abundance and breeding system among tropical forest species: Do dioecious species exhibit compensatory reproduction or abundances? J. Ecol. 97: 555–566.

Rosumek, F. B., F. A. O. Silveira, F. S. Neves, N. P. U. Barbosa, L. Diniz, Y. Oki, F. Pezzini, G. W. Fernandes, and T. Cornelissen. 2009. Ants on plants: A meta-analysis of the role of ants as biotic defenses. Oecologia 160: 537–549.

Schupp, E. W., and D. H. Feener. 1991. Phylogeny, lifeform, and habitat dependence of ant-defended plants in a Panamanian forest. In C. R. Huxley, and D. F. Cutler (Eds.). Ant-plant interactions, pp. 175–197. Oxford University Press, Oxford, U.K.

Silvertown, J. 2004. Plant coexistence and the niche. Trends Ecol. Evol. 19: 605–611.

Soepadmo, E., K. M. Wong, L. G. Saw, R. C. K. Chung, and R. Kiew. 1995–2006. Tree flora of sabah and sarawak. Forest Institute of Malaysia and Malaysian Nature Society, Kuala Lumpur, Malaysia.

Stevens, P. F. 2001. Angiosperm Phylogeny Website. Version 12, July 2012 [and more or less continuously updated since]. URL: http://www.mobot.org/MOBOT/research/APweb/.

Swaine, M. D., and T. C. Whitmore. 1988. On the definition of ecological species groups in tropical rain forests. Vegatatio 75: 81–86.

Valencia, R., R. B. Foster, G. Villa, R. Condit, J. C. Svenning, C. Hernández, K. Romolerouz, E. Loso, E. Magard, and H. Balslev. 2004. Tree species distributions and local habitat variation in the Amazon: Large forest plot in eastern Ecuador. J. Ecol. 92: 214–229.

Webb, C. O., D. D. Ackerly, and S. W. Kembel. 2001–2009. Phylocom - analysis of ecological communities and species traits, with phylogenetic tools. version 4.2.

Webb, C. O., and M. J. Donoghue. 2005. Phylomatic: Tree assembly for applied phylogenetics. Mol. Ecol. Notes 5: 181–183.

Weber, M. G., and A. A. Agrawal. 2014. Defense mutualisms enhance plant diversification. Proc. Natl Acad. Sci. USA 111 (46): 201413253.

Weber, M. G., and K. H. Keeler. 2013. The phylogenetic distribution of extrafloral nectaries in plants. Ann. Bot. 111: 1251–1261.

Welden, C. W., S. W. Hewett, S. P. Hubbell, and R. B. Foster. 1991. Sapling survival, growth, and recruitment: Relationship to canopy height in a Neotropical forest. Ecology 72: 35–50.

Wikstrom, N., V. Savolainen, and M. W. Chase. 2001. Evolution of angiosperms: Calibrating the family tree. Proc. R. Soc. Lond. Ser. B Biol. Sci. 268: 2211–2220.

Wright, J. S. 2002. Plant diversity in tropical forests: A review of mechanisms of species coexistence. Oecologia 130: 1–14.