

Divergence and diversity in the defensive ecology of *Inga* at two Neotropical sites

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Summary

1. Tropical plants express multiple defensive traits that enable them to resist, deter or escape herbivores. In this study, we approached plant defensive adaptations by focusing on variation and interaction between sets of plant defensive strategies from a community perspective.
2. We compared two sites: the aseasonal Amazonian forest of Yasuni, Ecuador, and the seasonal forest of Barro Colorado National Monument (BCNM), Panama. We suggest that differences in ant abundance between these sites influence the divergence, local assembly and interaction of chemical and indirect ant-mediated defences in 18 tree species of the genus *Inga* (Fabaceae: Mimosoideae).
3. Relative ant abundance and visitation to extra floral nectaries were significantly higher at Yasuni than at BCNM. Correspondingly, Yasuni *Ingas* had low leaf extract bioactivity compared with BCNM *Ingas*, suggesting that Yasuni *Ingas* rely more on indirect ant defences, while BCNM *Ingas* rely more on chemical defences.
4. Yasuni *Ingas* varied widely in their ant visitation and extract bioactivity, and the strategies were negatively correlated in a trade-off like pattern. In comparison, BCNM *Ingas* had a positive correlation and little variation in their ant visitation, suggesting that their defence ecology was driven by environmental characteristics other than ants.
5. An experiment planting the same *Inga* species at both sites supported the prediction of higher herbivore pressure at Yasuni than at BCNM. However, leaf damage to naturally occurring *Ingas* was similar between sites, suggesting that the adaptations at the two sites were equally effective. Damage levels were also similar among species with contrasting defensive strategies, suggesting that ant and chemical strategies were also equally effective at a single site.
6. *Synthesis*. While tropical forests are thought to be a cradle of specialized interactions that promote diversification and allow the coexistence of a large number of species, the observed divergence in the defence ecology of *Inga* between these two sites suggests that general community characteristics can also promote diversity by favouring local and geographical diversity in defensive solutions.

Key-words: ant defences, chemical defences, community interactions, defence assembly, extra-floral nectaries, herbivore pressure, herbivory, indirect defences, *Inga*, seasonality

Introduction

The ecological interactions between plants and their herbivores have led to the evolution of a great diversity of plant defences. Tropical plants express a variety of traits that enable them to intoxicate, resist, deter or escape herbivores (Coley 1983; Janzen & Waterman 1984; Aide 1993; Coley & Kursar 1996). The study of plant defences is commonly approached using

the framework of co-evolution (Ehrlich & Raven 1964). Co-evolution was originally envisioned as a process of mutual directional adaptations between a specialized herbivore and its preferred host. However, interaction webs are rarely so uncomplicated (Iwao & Rausher 1997; Morris & Lewis 2002; Novotny *et al.* 2004). More recent approaches to co-evolution address other important factors such as the role of trophic interactions (e.g. Dyer *et al.* 2001) and geographical variation in the intensity of co-evolutionary interactions (Thompson 2005). Plant defence studies often focus on the variation of individual defences following specific co-evolutionary

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interactions. Here we use a community approach to study how suites of defensive strategies may vary between sites.

Probably due to trade-offs in resource allocation, particular species express only a subset of the array of strategies potentially available to them (Kursar & Coley 2003). Among defensive trade-offs, the interaction between chemical and ant-mediated defences has been widely studied (Rehr *et al.* 1973; Dyer *et al.* 2001; Heil *et al.* 2002). However, it is possible that these trade-offs are also influenced by other characteristics of the community. For example, ant defences are known to be more prevalent in places where ants are more abundant (Bentley 1977; Koptur 1985; Barton 1986). Similarly, phenological strategies to avoid herbivores could be more effective in seasonal forests where herbivore pressure fluctuates throughout the year (Aide 1993; Martel & Kause 2002). In this paper we approach between-site variation in plant defence strategies by exploring the assembly of defences expressed by multiple species in a community. We define defence assembly as the suite of defences found at a site and examine how community characteristics may constrain the range of strategies expressed. We suggest that community traits could influence not only which strategies are possible, but also the interaction among defence strategies at a site.

We study the role of environmental variables in the defence divergence between two communities by focusing on the Neotropical genus *Inga* (Fabaceae: Mimosoideae). We compared two Neotropical sites: the species-rich, aseasonal forest of Yasuni in Ecuador, and the less-diverse, seasonal forest of Barro Colorado National Monument (BCNM) in Panama. *Inga* species express a wide array of defensive strategies, including indirect, chemical, developmental and phenological defences (Coley *et al.* 2005). However, for this study we measured only two types of defences: chemical and ant defences. Our approach provides evidence that differences in key environmental parameters between two communities can influence the assembly of defensive strategies in trees of the genus *Inga*.

ENVIRONMENT, DEFENCES AND BIOTIC INTERACTIONS IN *INGA*

Inga (Fabaceae: Mimosoideae) is a widespread, diverse, recently radiated and common component of the tropical flora (Pennington 1997; Richardson *et al.* 2001). The most characteristic defence in *Inga* is probably the presence of nectaries on the leaves that attract ant defenders (Koptur 1984). *Inga* species also produce a large variety of secondary chemicals with antiherbivore properties (Pennington 1997; Lokvam *et al.* 2004; Coley *et al.* 2005; Lokvam & Kursar 2005; Lokvam *et al.* 2006a,b). Some species have other morphological (e.g. trichomes) and phenological (e.g. synchrony in leaf flush) traits that can also act as defences (Coley *et al.* 2005). Ant and chemical defences, as well as the phenological characteristics of young leaves, are highly polymorphic within the genus, suggesting that these adaptations are evolutionarily labile. Despite *Inga* sympatric diversity, recent diversification and diversity in defensive strategies,

herbivores feeding on young leaves within the genus do not show a simple pattern of host specialization (Kursar *et al.* 2006).

Environmental and biotic differences between the sites should influence herbivore pressure and ant abundance. We predicted that herbivores and ants should be more abundant at aseasonal Yasuni than at BCNM based upon the observation that seasonal forests such as BCNM show a decrease in insect activity during the dry season (Wolda 1978). The realized levels of herbivory observed in *Ingas* at each site should be a function of the local herbivore pressure modulated by the presence of ants and the local defensive adaptations of the plants. If herbivore pressure is higher at Yasuni, herbivory levels should be higher unless local *Ingas* have evolved more effective defensive adaptations. We looked at ant and chemical defences and specifically asked the following questions. Is there support for our prediction of higher ant abundance and herbivore pressure at Yasuni than at BCNM? If so, do defensive adaptations of the local *Inga* species at the sites correlate with these differences? Finally, how are defensive strategies assembled within each community and how do they influence herbivory?

Methods

LOCATION AND CLIMATE

Most of our work was carried out in and around the Center for Tropical Forest Science's 50-ha Forest Dynamic Plots in Yasuni and BCNM. We used the plot census data (Hubbell *et al.* 1995; Valencia *et al.* 2004b) to characterize local *Inga* species abundance and composition (see Tables S1 and S2 in Supplementary Material).

Yasuni is located at 0°40' S, 77°24' W, 230 m a.s.l., in the Yasuni National Park and Biosphere Reserve, Ecuador. The park and adjacent Huaorani territory comprise 1 600 000 ha of mostly unbroken Amazonian rain forest. While human disturbance in the study site is low, the field station is crossed by a low-traffic road built by the oil companies and subsistence hunting by the local Huaorani inhabitants is common. The station is located in the north-western corner of the park, in terra-firme, mature forest bordering the Tiputini River. The 50-ha plot lies along two smaller ridges dominated by red clays and separated by a valley characterized by brown or grey alluvium. The difference between the lowest and the highest point of the plot is 33.5 m. The climate is aseasonal, the mean annual rainfall is 2830 mm and no month has less than 100 mm of average precipitation. The average daytime temperature in the shade is 25.2 °C (Romoleroux *et al.* 1997). In 2004, at the time of our study, only 25 ha had been mapped completely. The species counts in 25 mapped hectares place Yasuni as one of the forests with the highest alpha-diversity in the world (Valencia *et al.* 2004a). The field station and the plot are managed by the Pontificia Universidad Católica del Ecuador.

Barro Colorado National Monument (BCNM) is a 6500-ha reserve comprised of the Barro Colorado Island (BCI) and its surrounding mainland peninsulas: Bohío, Buena Vista, Peña Blanca and Gigante. BCI is a 1500-ha artificial island in the Gatun Lake, created when the Panama Canal was flooded in 1914. The vegetation is typical of a lowland semi-deciduous moist tropical forest. While part of the field station contains secondary forest that has been recovering since 1923, all our measurements were carried out in the 50-ha plot, on the plateau of the island, and other old-growth parts of the forest. The island is located at 9°09' N, 79°51' W and its highest

altitude is 130 m a.s.l. The climate of the area is seasonal. The average total yearly rainfall is 2600 mm, 90% of which falls during the rainy season from May to December, and the average daily temperature is 27 °C (Leigh *et al.* 1996). The plot was established by the Center for Tropical Forest Science of the Smithsonian Tropical Research Institute.

ANTS AND HERBIVORE PRESSURE AT YASUNI AND BCNM

Relative abundance of nectar-feeding ants

Differences in the relative abundance of nectar-feeding ants between the two sites were measured using pitfall sugar traps. Trapping was done during the rainy season at each site, because rainy seasons represent peaks of leaf production and ant abundance. Traps consisted of 10-mL plastic test tubes. In each trap we placed 4 mL of nectar prepared by dissolving 50 g of kitchen sugar and 2 mL of laundry soap in 1000 mL of 5% ethanol solution (method adapted from Feener & Schupp 1998). Traps were placed in the understorey of old-growth forest, along the trails, throughout the study site. Because we were interested in ants that would climb or live on saplings and trees, we tied the traps to stems of plants at different heights that could be reached by hand. Traps were left in the field for 48 h during four trapping days at each site. There were additional trapping days in both sites that were discarded because rainy weather affected trapping. On each trapping day we placed 30 traps in BCI and c. 50 traps in Yasuni. More traps were placed in Yasuni than in BCNM because we were trying to account for the higher topographic heterogeneity (ridges and valleys) at this site. However, because the results showed no effect of topography on ant trapping numbers (T. Brenes-Arguedas, unpublished data) the Yasuni data were combined. In total we placed 210 traps at Yasuni (during March and April 2004) and 120 traps at BCNM (during May and June 2004). Ant abundance was measured as the average number of ants collected per trap. Ants were classified by morphospecies and identified to subfamily and genus. We classified the different morphospecies as group or individual foragers based on collection data and field observations. For the purpose of this paper we defined group foragers as those morphospecies that were regularly found in groups of two or more ants on the leaves or in the traps.

Herbivore pressure on Inga

Herbivore pressure was measured using a reciprocal transplant experiment with *Inga spectabilis*. Ideally this would have been tested with more than one *Inga* species, but collection, transport and germination of live seeds of wild species between countries proved impractical. *I. spectabilis* has a cultivated, edible fruit easily acquired in local markets throughout meso- and South America and grows naturally, though at low abundance, in both study sites. We acquired *I. spectabilis* seeds in Ecuador (from Esmeraldas) and in Panama (from La Chorrera) and transported half to Yasuni and half to BCNM field stations. Seeds were germinated in screened shade-houses to avoid seed predation. However, the Ecuadorian seeds at Yasuni died during germination due to a fungus; thus we planted Ecuadorian seedlings only in BCNM.

Half the Panamanian seedlings were randomly assigned to an ant exclusion treatment. Ants were excluded from the plants by applying a coat of insect trap (Tangle-Trap, The Tanglefoot Company, Michigan, USA) directly on the stem. At Yasuni, we planted 75

plots each with two Panamanian seedlings, one control and one with ant exclusion treatment. Plots were placed 5–15 m apart from each other along trails, outside the buffer zone of the 50-ha plot. At BCNM, we planted 63 plots, each with three seedlings: one Ecuadorian, one Panamanian control and one Panamanian with ant-exclusion treatment. Plots were placed 5–50 m from each other in random locations throughout a 2-ha section of the forest on Buena Vista Peninsula, roughly 4 km away from the 50-ha plot. The gardens were started on July 2004 and harvested 10 months later. Once a month, we measured herbivory as percentage of area damaged on freshly expanded leaves using a grid and annotated the presence of ants in the nectaries.

At the end of the experiment we measured standing damage (as percentage of leaf area) in all mature leaves of the seedlings. We also did a single-point census of herbivores (including homopterans, lepidopterans and orthopterans) as a function of leaf area. This was done by first looking for insects before touching the plants and then carefully turning over the leaves. For comparison we also counted lepidopteran herbivores on naturally growing *Inga* in the two sites during the herbivory measurements described in the next section. These censuses are used only as observational data, as they represent only one-point estimates, and no attempt was made to correct for the presence of ‘tourists’ (herbivores that are not feeding on the plant).

Leaf damage to naturally growing Inga species

Leaf damage measurements focused on 14 focal species. Based on data found in Hubbell *et al.* (1995) and Valencia *et al.* (2004b), these species represent 67% of the *Inga* stems in the Yasuni plot and 86% of the *Inga* stems in the BCNM plot. Five of the focal species were shared between the two sites: *Inga marginata*, *ruiziana*, *punctata*, *nobilis* and *umbellifera*. Six species were exclusive to Yasuni: *Inga auristellae*, *capitata*, *umbratica*, *acreaana*, *leicalycina*, and one species that is yet to be described (census name: morphospecies ‘6cuadra’). Three species were exclusive to BCNM: *Inga goldmanii*, *acuminata*, *sapindoides*. *Inga* is a taxonomically difficult genus; species names and identification are based on those used by the first plot census in Yasuni (Valencia *et al.* 2004b) and the 1995 census at BCNM (Hubbell *et al.* 1995).

Leaf damage of the focal species was measured as standing damage on mature leaves. The trees measured included any individual within the study area greater than 50 cm tall, whether growing in the understorey or in gaps. Individuals were excluded only if their foliage was not accessible from the ground. Thus, average damage for our species is a site mean representing mostly understorey herbivory. For each individual plant, we selected one random branch and averaged the percentage area damaged using a grid in at least three mature leaves starting from the tip. More than three leaves were measured in small-leaved species with dense foliage, in an attempt to sample a more representative part of the branch. Mature leaf damage underestimates herbivory in instances when whole leaves were consumed during expansion, but it has the advantage that, for long-lived leaves, it can include herbivory events from previous seasons.

DEFENSIVE ADAPTATIONS

Defensive adaptations were measured for 18 focal species. These include the same focal species used for the leaf damage measurements, plus four other *Inga* species exclusive to BCNM: *pezizifera*, *cocleensis*, *thibaudiana* and *laurina*.

Ant visitation to young *Inga* leaves

Inga rewards for ants are limited to extra-floral nectaries present in the rachis of the leaf. Because these glands only produce nectar during the period of leaf expansion and ants on mature leaves are rare, only young expanding leaves were measured. Ant visitation was analysed in two forms, as the average number of ants per flush of expanding young leaves and as ant presence/absence in young leaf flushes. Ant visitation data at BCNM were collected throughout the year from 2001 to 2004 as part of a long-term project, while at Yasuni they were counted only during the rainy season of 2004 and 2005.

Bioactivity of young leaf extracts

Young (5–80% of full expansion) leaves were collected from at least three individuals per focal species per site. The fresh leaves were macerated in 95% EtOH on the day of collection, frozen and later transported to the University of Utah for extraction. Extracts were prepared by successively extracting the insoluble leaf materials with 80% EtOH, 70% acetone, dichloromethane and water, and recombining all the concentrated fractions (Coley *et al.* 2005).

The antiherbivore effect of the extracts of the different focal species was tested using feeding trials on the larvae of *Heliothis virescens* (Lepidoptera: Noctuidae), a generalist herbivore of tropical origin. The use of a generalist herbivore is important because our aim is the study of general defence strategies and not the observation of specific co-evolutionary interactions. Native *Inga*-feeding herbivores are likely to show resistance to particular plant metabolites depending on the subset of species consumed in the wild. In contrast, the response of a generalist such as *H. virescens* to *Inga* extracts is a better indicator of the differences among *Ingas* in a key trait: their capacity to deter the majority of a highly diverse polyphagous or oligophagous herbivore assemblage.

Bioactivity was measured as Growth Inhibition 50 (GI₅₀): the concentration at which larvae grow 50% as large as larvae reared on control diets. GI₅₀ was calculated by measuring larval weight after 8 days of feeding on an artificial agar diet with different concentrations of plant extracts. Diet preparation, assay methods and data analysis are described in detail in Coley *et al.* (2005) and the GI₅₀s of some of the BCNM focal species have been reported before (Coley *et al.* 2005; Brenes-Arguedas *et al.* 2006).

Community assembly of defence strategies

While some of the focal species were shared, our two study sites did not have the same species composition. Thus, the site means for each defensive strategy are partly a result of the local *Inga* species

assembly and the specific defence strategies expressed by those species. To visualize the variation in defensive adaptations among *Inga* species within each community, we plotted extract bioactivity as a function of ant visitation for the most abundant *Inga* species from Yasuni and BCNM.

DATA ANALYSIS

Statistical analyses were performed using SAS (The SAS system, v8.1). Count data were analysed with negative-binomial generalized linear models (SAS proc genmod). Presence/absence data were analysed using logistic regression (SAS proc logistic). Other comparisons were analysed with mixed-effect or simple linear models (SAS proc glm). Specific models are described in the Results.

Results

ANTS AND HERBIVORE PRESSURE AT YASUNI AND BCNM

Relative abundance of nectar-feeding ants

Ant trapping was higher at Yasuni than at BCNM (Table 1). On average, traps at Yasuni collected twice as many ants as at BCNM (Table 1; generalized linear model with negative-binomial distribution, $z = 4.5$, $P < 0.0001$). Higher collection rates in Yasuni were partly due to more traps collecting ants (Yasuni = 50%, BCNM = 38%) and partly to more ants collected per trap (percentage of traps with two or more ants: Yasuni = 26%, BCNM = 11%). Forty-five per cent of the morphospecies collected at Yasuni and 33% of the morphospecies at BCNM were classified as group foragers. Because group foragers were often collected as multiple individuals of the same species per trap, we corrected for pseudo-replication by calculating species abundance only with presence/absence data of ant morphospecies per trap. Group foragers were more abundant at Yasuni than at BCNM (percentage of traps with at least one group forager morphospecies: Yasuni = 74%, BCNM = 21%). Ant species richness was also higher at Yasuni. The number of morphospecies per trap was not significantly different between the sites (Table 1; negative-binomial glm, $P = 0.43$), but in 120 traps we accumulated nearly twice as many morphospecies at Yasuni than at BCNM. This is partly a result of higher capture rates and partly a result of higher ant diversity in Yasuni. Yasuni had neither a single dominant morphospecies nor a single dominant

Table 1. Comparison of pitfall-trap ant collections between the two sites

	Yasuni	BCNM
1. Number of ants per trap	1.4	0.54*
2. Number of morphospecies per trap	1.3	1.1
3. Most abundant morphospecies (abundance)	<i>Pseudomyrmex</i> sp1. (14%)	<i>Ectatomma ruidum</i> (42%)
4. Most abundant genera (abundance, number of morphospecies)	<i>Camponotus</i> (18%, 9) <i>Crematogaster</i> (16%, 7) <i>Pseudomyrmex</i> (16%, 3)	<i>Ectatomma</i> (42%, 1)

*Statistically significant difference at $P < 0.01$.

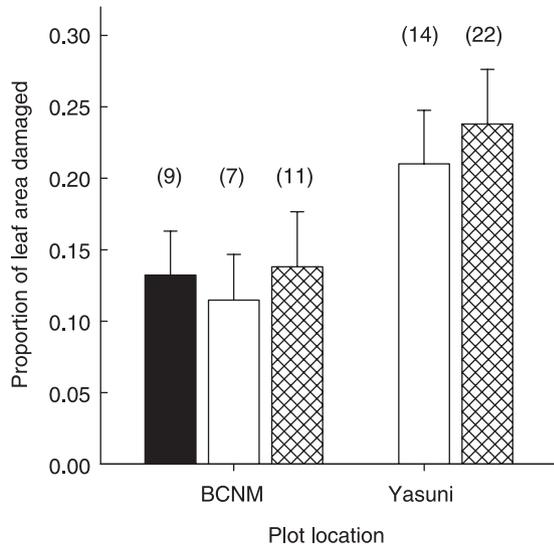


Fig. 1. Leaf area damaged by herbivory on recently expanded leaves of *I. spectabilis* seedlings planted at BCNM or at Yasuni. Seedlings from Ecuador with normal ant visitation (black), and from Panama with normal ant visitation (white) or with ant-exclusion treatment (hatched). Error bars are standard errors. The numbers in parenthesis above the bars represent the number of insect herbivores per metre square of *I. spectabilis* leaf area in each of the treatments from a single count at the time of the harvest.

genus of nectar-feeding ant. The most abundant morphospecies represented only 14% of the collections (Table 1), and three different genera with similar abundances represented 50% of the collections (Table 1). These three genera comprised 19 different morphospecies. In contrast, the BCNM nectar-feeding ant community was dominated by one species, *Ectatomma ruidum*, which represented more than 40% of the collections (Table 1). This species also represented 57% of the observations in young-leaf ant counts.

Herbivore pressure on *Inga*

I. spectabilis seedlings at Yasuni suffered nearly twice the level of herbivory on young leaves as seedlings growing at BCNM (Fig. 1; mixed-effect ANOVA with plot as random blocking factor, site effect: $F = 6.61$, $P = 0.011$). Ecuadorian seedlings growing at BCNM experienced the same herbivory levels as their Panamanian neighbours ($F = 1.65$, $P = 0.20$), suggesting that the higher damage at Yasuni was independent of the genetic background of the seedlings. At the end of the experiment, the standing damage present in the mature leaves of the *I. spectabilis* seedlings was also higher at Yasuni than at BCNM (Yasuni = 30%, BCNM = 24%; $F = 5.41$, $P = 0.021$).

Herbivore counts also supported higher herbivore pressure at Yasuni, keeping in mind that they were single-point estimates and cannot be compared statistically. At the time of harvest, herbivores were more abundant on the *I. spectabilis* seedlings at Yasuni than at BCNM (Fig. 1). Also consistent with the above results, the count of lepidopteran larvae on

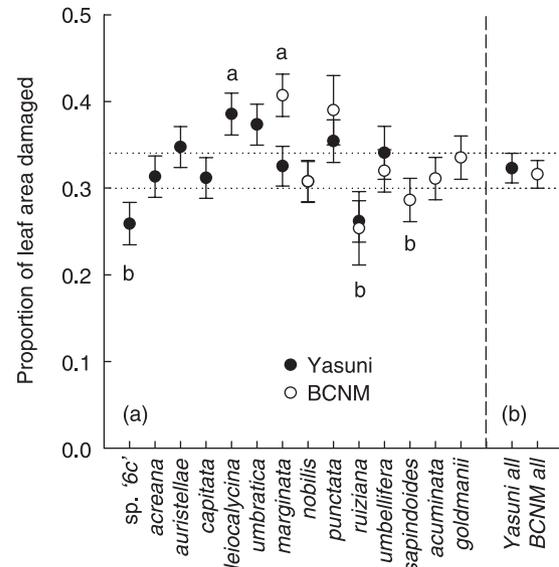


Fig. 2. Accumulated leaf area damage in mature leaves of *Inga* species at BCNM and Yasuni averaged by species (a) and by site (b). Error bars are standard errors. Species with different lower case letters have significantly different ($P < 0.05$) damage levels based on Tukey comparisons within site ('ab' in all other species was omitted for clarity). Dotted lines are the 95% confidence interval for the mean of all species.

focal *Inga* species growing naturally in the 50-ha plots was also higher at Yasuni (0.24 larvae per m² of leaf area) than at BCNM (0.11 m⁻²).

Ant exclusion on *I. spectabilis* seedlings did not significantly influence herbivory in either of the two sites (Fig. 1; mixed-effect ANOVA, treatment effect: $F = 0.52$, $P = 0.47$). However, this result may be due to the particular characteristics of the *I. spectabilis* seedlings, and not to the actual experimental treatment. Ant visitation to young leaves of control *I. spectabilis* seedlings (0.2 ants per flush) was considerably lower than the visitation to any of the focal *Inga* species (see below). These levels of visitation were probably not sufficient for defensive purposes

Leaf damage to naturally growing *Inga* species

Within each site there were significant differences in standing leaf damage present in the mature leaves of the focal *Inga* species (ANOVA; Yasuni, $F = 2.44$, $P = 0.0018$; BCNM, $F = 2.71$, $P = 0.0069$). However, most species did not differ among themselves, and only a few species had significantly higher or lower damage levels (Fig. 2a). The two sites did not differ in the average standing damage (two-way ANOVA, site effect, $F = 0.07$, $P = 0.79$, Fig. 4b). When comparing the species shared between the two sites (Fig. 2a and Table S2), two species had non-significantly higher damage levels in Yasuni, one was equal, and two had higher damage levels at BCNM than at Yasuni (*I. marginata* significantly so, $P = 0.015$). The lack of difference between the sites was not explained by the shortcomings of measuring mature leaves, as the *I. spectabilis*

seedlings of the reciprocal transplant experiments differed significantly in the standing damage of their mature leaves by 6%.

DEFENSIVE ADAPTATIONS

Ant visitation to young Inga leaves

Consistent with the pitfall-trap results (Table 1), ant visitation to young *Inga* leaves was higher on average at Yasuni than at BCNM (ants per flush at Yasuni = 4.00, at BCNM = 1.90; generalized linear model with negative-binomial distribution $\chi^2 = 81.10$, $P < 0.0001$). At BCNM, rainy seasons tended to have slightly but significantly more ant visitation than dry seasons (rainy = 1.9 ± 0.1 , dry = 1.19 ± 0.09 ; $\chi^2 = 32.4$, $P < 0.0001$), and 2004 had significantly higher ant visitation than the previous 3 years (ants per flush 2001–03 = 1.4, 2004 = 2.5; $\chi^2 = 42.3$, $P < 0.0001$). However, ant visitation was still higher at Yasuni even when comparing it with the BCNM 2004 rainy season ($\chi^2 = 10.32$, $P = 0.0013$). The difference in ant visitation between the two sites was also significant when conservatively comparing only ant presence/absence data (Yasuni = 0.64, BCNM 2004 = 0.58; logistic regression, $\chi^2 = 4.76$, $P = 0.029$).

Within each site, different species had significantly different ant visitation levels. With 4 years of ant visitation data at BCNM, the differences in ant visitation among species were highly significant (Kruskal–Wallis, $n = 2196$, $\chi^2 = 176$, d.f. = 10, $P < 0.0001$). The differences in the probability of ant presence were also highly significant (logistic regression, $\chi^2 = 129.5$, $P < 0.0001$). At Yasuni, despite having fewer data points, differences among species were also significant for ant counts (Kruskal–Wallis, $n = 269$, $\chi^2 = 18.4$, d.f. = 10, $P = 0.05$) and for ant presence/absence (logistic regression, $\chi^2 = 21.9$, $P = 0.03$). While ant visitation is not a direct measurement of defence investment, species with locally high ant visitation probably represent species with better ant rewards, possibly associated with the quality or quantity of the nectar. Nectar quantity can be controlled by the amount of nectar per gland or by the number of glands per leaf. The number of extra-floral nectaries per leaf varies between two and eight among species. At BCNM there was a weak non-significant trend for higher ant visitation to the *Inga* species that had more nectaries in the leaves, but this was not observed in Yasuni (data not shown).

Bioactivity of young leaf extracts

As a naïve generalist herbivore, *H. virescens* was extremely sensitive to the secondary chemicals present in our extracts. Its growth was reduced by extract concentrations between 1% and 5% of the dry weight of the bioassay diet. Those concentrations are an order of magnitude lower than the concentrations normally observed in young *Inga* leaves (20–50% of dry weight).

As we have only one GI_{50} estimate per species, we based our statistical comparisons on the standard error of the estimates. We compared the average *Inga* bioactivity between sites using mixed-effect ANOVA, with species as a random factor and the inverse of the standard error of the GI_{50} estimates as the

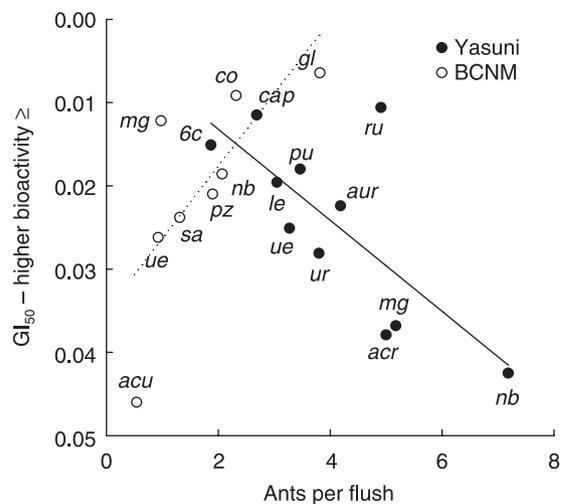


Fig. 3. The relationship between the average ant visitation and the leaf extract bioactivity (GI_{50}) of the focal *Inga* species at Yasuni (linear regression, $r^2 = 0.47$, $P = 0.01$, solid line) and BCNM ($r^2 = 0.44$, $P = 0.04$, dotted line). Labels for *Inga* species are *acr* = *accreana*, *acu* = *acuminata*, *aur* = *auristellae*, *cap* = *capitata*, *co* = *cocleensis*, *gl* = *goldmanii*, *mg* = *marginata*, *nb* = *nobilis*, *pu* = *punctata*, *pz* = *pezizifera*, *ru* = *ruiziana*, *sa* = *sapindoides*, *ue* = *umbellifera*, *ur* = *umbratica*, *6c* = unnamed species.

weight. This method places less importance on values for which the GI_{50} is not known with accuracy (large standard error) and corrects for shared species between the sites. On average, leaf extracts from Yasuni *Ingas* were less bioactive (higher GI_{50}) than leaf extracts from BCNM (average GI_{50} at Yasuni = $2.5 \pm 0.3\%$, BCNM = $1.7 \pm 0.2\%$; $F = 6.28$, $P = 0.046$). Shared species were compared using the standard errors of the estimates to calculate *t*-values. Two species had significantly higher bioactivity in BCNM leaves than in Yasuni leaves (*I. marginata*, $P = 0.001$, and *I. nobilis*, $P = 0.03$).

Community assembly of defence strategies

The range of ant visitation numbers per species was different between the two sites (Fig. 3 and see Table S2). At BCNM, ant visitation ranged from 0.5 to 3.8 ants per flush (4.9 if we include rare *I. ruiziana*). At Yasuni, average ant visitations ranged from 1.9 to 7.2 ants per flush, a larger range, with a greater number of ants per flush than at BCNM. Leaf extract bioactivity varied considerably among species within both sites, and the range was similar in both sites. At Yasuni GI_{50} ranged from 1.1 to 4.3 and at BCNM from 0.6 to 4.6. At Yasuni there was a significant negative correlation between bioactivity and ant visitation (Fig. 3). Bioactivity was lower (high GI_{50}) in species where ant visitation was high. At BCNM the trend was in the opposite direction: bioactivity of the extracts was lower when the ant visitation was also lower (Fig. 3). None of the variables measured (ant visitation, extract toxicity or species abundance, nor their combination) explained the observed levels of standing damage for each species (Wilks $\lambda = 0.86$, $P = 0.45$).

Discussion

Yasuni and BCNM differ in the seasonality of the rains, as well as the diversity and density of *Inga* tree species. Based on these differences we predicted that *Inga* trees would have higher herbivore pressure and ant abundance at Yasuni than at BCNM. Our results support those predictions and also support the hypothesis that the assembly of defensive strategies of the local *Inga* species can be partially explained based on those differences.

ANTS AND HERBIVORE PRESSURE AT YASUNI AND BCNM

There was a substantial difference in the ant community between the two sites. We found higher abundance of nectar-feeding ants in Yasuni measured with the pitfall-trap collections (Table 1) and with the ant census on young leaves. The ant community in Yasuni was characterized by a greater number and diversity of ants and by a higher abundance of aggressive, group-foraging morphospecies (Table 1). The nectar-feeding ant community at BCNM was instead dominated by a single species, *Ectatomma ruidum*, which is usually found alone on the leaves. Ant presence/absence (the probability of finding at least one ant on a plant with at least one young leaf) was also significantly higher in Yasuni, indicating that higher ant visitation was not driven solely by the higher number of foragers per leaf. The temporal analysis of ant visitation at BCNM *Ingas* indicated that ants were more abundant during the rainy season. We were unable to measure seasonal trends at Yasuni, but due to the lower seasonality, more constant ant abundance is a reasonable expectation there.

We had predicted that herbivore pressure on *Inga* should also be higher at Yasuni than at BCNM. This prediction was based on the common hypothesis that aseasonal forests have higher herbivore and pathogen pressures (Marquis & Braker 1994). The results of the *I. spectabilis* reciprocal transplants (Fig. 1) and of the herbivore counts support this prediction. The *I. spectabilis* gardens showed that when plants from the same genetic background were planted in both sites, those growing in Yasuni experienced more herbivory. This conclusion is based on only one species, but given the low specialization of herbivores within the genus *Inga* (Kursar *et al.* 2006), it could reflect the herbivore pressure experienced by other *Inga* species as well. If indeed there is higher herbivore pressure for all Yasuni *Ingas*, its impact was not reflected in the standing damage of the focal *Inga* species growing naturally in the 50-ha plots. Yasuni and BCNM *Ingas* had on average the same levels of standing damage (Fig. 2b), as did four out of the five species that are shared between the two sites (Fig. 2a and Table S2). This lack of a difference may be an indication that Yasuni plants are able to balance higher herbivore pressure with more effective defences, but this conclusion remains to be confirmed by additional measures of herbivore pressure and herbivory.

DEFENSIVE ADAPTATIONS

The differences between the two ant communities suggest that ant visitation is a more reliable resource at Yasuni than at BCNM. Higher ant abundance in Yasuni translated into higher visitation probability and leaf visitation numbers. Thus, based on the assumption that more ants patrolling the leaves translates into lower opportunities for the herbivores, we conclude that ants are a better defence at Yasuni than at BCNM. We attempted to quantify the local efficiency of ants in modulating herbivore damage at each site using the *I. spectabilis* reciprocal transplant experiments. However, seedlings of *I. spectabilis* experienced unusually low visitation by ants, and exclusion had little effect on leaf damage (Fig. 1). It is possible that this species is among those with poor ant rewards (Fig. 3), or that the low leaf production of small seedlings may not provide enough incentive for ants to remain and defend the resource. It is important to stress that our conclusion of better ant defences in Yasuni does not necessarily imply that *Inga* species at Yasuni invest more in ant rewards than at BCNM. When comparing *Inga* species within one site, differences in ant visitation among species could represent a relative measure of the quality or quantity of the ant rewards. Yet, because ant abundance was also higher at Yasuni, it is equally likely that the average ant reward was the same at both sites and that differences in recruitment were due solely to differences in local ant abundances.

According to our bioassays with *H. virescens*, *Inga* species at BCNM produce better chemical defences. The extracts of *Inga* species from BCNM were on average more bioactive than those from Yasuni. Note that our measure of chemical defences, bioactivity against a generalist herbivore, is similar to the ant defences in that it is a broad spectrum strategy aimed against the majority of a highly diverse herbivore assemblage. The secondary compound profile of different *Inga* species is fairly distinctive (J. Lokvam, unpublished data) and secondary compound concentration varies considerably during leaf expansion (Brenes-Arguedas *et al.* 2006). Furthermore, the chemical profile of some shared species like *I. marginata* differs slightly between sites (T. Brenes-Arguedas, unpublished data). Thus, comparing chemical defences using bioassays allowed us to compare species with very different chemical profiles, where simple comparisons of extract concentrations would only have been meaningful for very specific co-evolutionary interactions.

Together, these measures of defensive strategies suggest a pattern of greater reliance on ants at Yasuni and on secondary metabolites at BCNM. At BCNM, possibly due to the low abundance of ants, *Inga* species rely mostly on better chemical defences. Conversely, *Inga* species at Yasuni appear to rely on ant-defence and have relaxed investments in chemical defences. Two of the species shared between sites had significantly lower bioactivity at Yasuni (*I. marginata* and *I. nobilis*; see Table S2), consistent with this proposition. Ant visitation levels cannot be compared directly between sites.

COMMUNITY ASSEMBLY OF DEFENCE STRATEGIES

The above general pattern is based on averages, but within each site there was large variability in defensive strategies among species (Fig. 3). The range of ant visitations among BCNM species had smaller values than the range of ant visitations among Yasuni species (Fig. 3). This is likely to be the result of the difference in ant abundance between the two sites. Instead, the range of bioactivities was very similar between the two sites (Fig. 3). The difference in mean bioactivity was the result of most BCNM species having low GI_{50} . In Fig. 3, BCNM species are plotted as a relatively tight cluster at the upper left corner of the graph, with the exception of *I. acuminata*. Instead, at Yasuni, *Ingas* display a more even distribution in their bioactivity and ant visitation values (Fig. 3). Some species had poor ant visitation and high extract bioactivity, similar to the species from BCNM, and other species had high ant visitation and low extract bioactivity, extending the axis of phenotypes to the lower right corner of Fig. 3. This suggests that while ants are a more reliable resource at Yasuni, not all *Inga* species there relied on them for their defence. Indeed, both phenotypes appear to be equally reliable strategies. For example, both *I. nobilis* and *I. capitata* had equal damage levels (Fig. 2), yet they were found in opposite corners of Fig. 3. In general, neither ant visitation nor extract bioactivity explained the variation among species in standing damage in either study site.

There were different correlations among defence strategies at each site. A negative correlation between ant visitation and extract bioactivity, such as the one observed in Yasuni, is consistent with a trade-off in plant investment between defence strategies. Our results are a multispecies comparison, and our measures of ant visitation and bioactivity may not represent true differences in investment. Yet, if this correlation does represent a trade-off, it appears to depend on the local ant abundance. Ants are pervasive in the Yasuni community, and most plants fell along this possible ant-chemical defence continuum. Ants are less abundant in the BCNM community and such a trade-off like pattern was not observed there. The defence assembly at BCNM may instead be driven by variables other than ants. A positive correlation would suggest that low ant visitation and bioactivity could be just as successful as heavy investment in both. For example, *I. acuminata* had very low bioactivity and ant visitation (Fig. 3), yet its damage levels were the same as those of the well-defended *I. goldmanii* (Fig. 2). However, *I. acuminata* has higher synchrony in leaf production than *I. goldmanii*. In seasonal BCNM, synchrony in leaf flushing for *Inga* species appears to be higher when bioactivity is lower (P. D. Coley and T. A. Kursar, unpublished data), suggesting that phenological defence strategies may be a more effective alternative to ants.

Finally, it is important to note that the defence assembly in these communities can result from two possible mechanisms that we have not attempted to separate here. One mechanism involves different selection pressures leading to the evolution of different adaptations at each site. We see evidence for this in the divergence between populations of *I. marginata* and

I. nobilis. However, for the most part, Yasuni and BCNM do not have the same *Inga* species composition. Hence, another explanation for the observed differences across sites is species assembly. Species assembly can be the result of geographical or distance factors. However, interaction between the environment and biotic pressures can also be an important determinant of plant distribution (Fine *et al.* 2004). Thus, it is also possible that ant abundance in Yasuni may have favoured the colonization by *Inga* species with good ant rewards and poor chemical defences (the lower right quadrant of Fig. 3) that would otherwise be excluded from sites like BCNM.

In conclusion, we have shown that defensive strategies of *Inga* species vary between sites, and we extracted general patterns and hypotheses to explain the observed assemblies of defensive strategies. *Inga* defences at Yasuni are probably driven by the characteristics of the ant community, and at BCNM possibly by the seasonality of the site. Often, alternative strategies were equally effective such that there was little variation in standing damage among *Inga* species and between sites, despite evidence suggesting differences in herbivore pressure. Our results do not reject the influence of specialized interactions and individualistic species responses in the observed patterns, but show that much of the variation in defensive strategies among congeners can be explained by general community traits. Thus, while tropical forests are thought to be a cradle of specialized interactions that promote diversification and allow the coexistence of a large number of species, diffuse community interactions may also promote plant diversification by favouring local and geographical divergence in defence solutions.

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Supplementary material

The following supplementary material is available for this article:

Table S1. *Inga* genus density and diversity in BCNM and Yasuni Forest Dynamics Plots

Table S2. Abundance, herbivory, ant visitation and bioactivity for the focal *Inga* species in BCNM and Yasuni

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