SPECIAL TOPIC: FROM PLANTS TO HERBIVORES

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Consequences of interspecific variation in defenses and herbivore host choice for the ecology and evolution of *Inga*, a speciose rainforest tree

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Abstract

We summarize work on a speciose Neotropical tree genus, *Inga* (Fabaceae), examining how interspecific variation in anti-herbivore defenses may have evolved, how defenses shape host choice by herbivores and how they might regulate community composition and influence species radiations. Defenses of expanding leaves include secondary metabolites, extrafloral nectaries, rapid leaf expansion, trichomes, and synchrony and timing of leaf production. These six classes of defenses are orthogonal, supporting independent evolutionary trajectories. Moreover, only trichomes show a phylogenetic signature, suggesting evolutionary lability in nearly all defenses. The interspecific diversity in secondary metabolite profiles does not arise from the evolution of novel compounds, but from novel combinations of common compounds, presumably due to changes in gene regulation. Herbivore host choice is determined by plant defensive traits, not host phylogeny. Neighboring plants escape each other's pests if their defenses differ enough, thereby enforcing the high local diversity typical of tropical forests. Related herbivores feed on hosts with similar defenses, implying that there are phylogenetic constraints placed on the herbivore traits that are associated with host use. Divergence in defensive traits among *Inga* appears to be driven by herbivore pressure. However, the lack of congruence between herbivore and host phylogeny suggests that herbivores are tracking defenses, choosing hosts based on traits for which they already have adaptations. There is, therefore, an asymmetry in the host–herbivore evolutionary arms race.

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Introduction

The interactions between plants and herbivores have profound ecological and evolutionary consequences for life on earth. This evolutionary arms race may promote speciation of both groups, and the antagonistic interactions may enhance ecological coexistence and diversity (Ehrlich and Raven 1964; Janz 2011; Marquis et al. 2016). In this review, we summarize our work on a speciose Neotropical tree genus, *Inga* (Fabaceae, Pennington 1997), examining how interspecific variation in anti-herbivore defenses may have evolved, how defenses shape host choice by herbivores and how they might regulate community plant composition and influence species radiations (Farrell et al. 1991; Becerra 1997; Futuyma and Agrawal 2009; Schemske 2009). *Inga* is an excellent system to explore plant–herbivore interactions in ecological and evolutionary time because the genus has explosively radiated in the last 4 million years, giving rise to over 300 species (Richardson et al. 2001). Furthermore, at any given Neotropical rainforest site, it is one of the most

diverse tree genera and one of the most abundant in terms of individuals (Valencia et al. 2004; Dexter et al. 2017). We have data on defensive traits and herbivores for > 100 *Inga* species at sites in Panama, Ecuador, Peru, Brazil and French Guiana (Table S1). The large number of closely related species and the broad geographic area allow comparative analyses and insights into the macroevolutionary patterns of defense and host associations.

Plants, pests and predators

Both top-down and bottom-up forces affecting herbivory are centered on young, expanding leaves. In *Inga*, as with most tropical trees, approximately 28% of the leaf area is lost to herbivores during the 1–3 week period from bud-break to full expansion (Coley 1980, 1983; Coley and Aide 1991; Kursar and Coley 2003; Fig S1). This represents more than 70% of the damage accrued during the entire lifetime of a leaf, typically 2–3 years (Coley and Aide 1991). Young leaves are particularly vulnerable to herbivores because growth requires high nitrogen and cell walls cannot be toughened until expansion is completed. As soon as leaves reach full size, they rapidly toughen, nitrogen content declines and as a consequence, herbivore attack rate drops by about 50-fold (Coley 1983; Kursar and Coley 2003; Brenes-Arguedas et al. 2006). Toughness is one of the most effective defenses, and most shade-tolerant species have mature leaves that are very tough. In contrast, expanding leaves have very diverse defenses that are poorly understood (see 'Defenses of *Inga*'), and it is these that we focus on.

In lowland tropical forests, Lepidoptera cause more damage to leaves than do other herbivores (Janzen 1988; Barone 1998; Novotny et al. 2004). Although *Inga* is associated with a great variety of herbivores in addition to Lepidoptera, including Coleoptera, Orthoptera, phloem-feeding Coreidae, Diptera, sawflies, and Phasmida, our studies confirm that Lepidoptera are the primary herbivores. In Peru, lepidopteran larvae comprise ~ 80% of all herbivore species sampled on saplings of *Inga*.

Although pathogen attack is a critical source of mortality in seedlings of many species, we hypothesize that insect herbivores are the main pest for saplings throughout the decades they persist in the understory. A 5-year study of 32 shade-tolerant species on Barro Colorado Island found an average of 25% leaf loss while leaves were expanding (Table 1). In contrast, an average of only 4.2% of the leaf area was damaged by pathogens, and this was primarily due to 100% loss of a few leaves. For 10 species of *Inga*, 26% of leaf area was lost while leaves were expanding, with 2.6% lost to pathogens. In contrast, the damage to mature leaves of nine species of *Inga* was only 0.23% (Table 1). Therefore, because expanding leaves

are constrained to be tender and nutritious, their defenses are critical traits for mediating plant–herbivore interactions and are under strong natural selection from insect herbivores.

Although expanding leaves are preferred, they are ephemeral. For larvae to complete development before the leaf toughens and becomes unpalatable, oviposition must occur at bud-break (Aide and Londoño 1989), making timely host location challenging for ovipositing females. Furthermore, many plant species in the understory do not continuously produce young leaves, but may have only a few flushes per year. Thus, we argue that, contrary to generalizations that competition between species is low and food availability is high (Hairston et al. 1960), herbivores that are dependent on expanding leaves of a restricted set of plant taxa may be food-limited (Kursar et al. 2006).

Critical for understanding why herbivores focus on ephemeral, expanding leaves instead of abundant, mature leaves are parasitism and predation. High rates of both place a premium on minimizing the length of the larval period when Lepidoptera are most vulnerable to natural enemies (Benrey and Denno 1997; but see Lill and Marquis 2001). In fact, caterpillars feeding on expanding vs. mature leaves grow 2.2 times faster (Coley et al. 2006). In a single site in Panama, 20.7% of 1225 late-instar lepidopteran larvae collected in the field from various phylogenetically disparate hosts were parasitized. Regarding predation, the risk increases towards the equator. Predation on clay caterpillars, often used as a measure of maximum rates for undefended caterpillars (Richards and Coley 2007), was 2–7.5%/day in temperate broad-leaved forests, 14%/day in Panama and increased to 29–45%/day in Central Africa and 35–73%/day in the Amazon (Table 2). Our latitudinal data are similar to a larger study (Fig. S1 in Roslin et al. 2017) that found approximately 7.5%/day for temperate forests and a maximum of 65%/day in a tropical forest. And as with our data, Roslin et al. (2017) reported that at all latitudes, birds accounted for only a small fraction of attacks, the majority being from arthropods, especially ants. Through 24-h observations of real caterpillars in Utah, 1.8% of them were eaten daily, very similar to the 1.9%/day observed for clay caterpillars (Fig. 1). For 24-h observations of real caterpillars in Panama, predation rates were 13.6%/day for species that fed on fast-expanding young leaves and appeared to have little defense, such as green geometrids and gelechiids. This rate is identical to the rate for undefended clay caterpillars confirming that clay models are a good estimate of predation risk. In summary, we hypothesize that the high potential rates of predation and parasitism explain why most herbivores feed on ephemeral expanding leaves, which permit faster larval growth.

Table 1 Percent leaf area lost for expanding and mature leaves

Species	Expanding leaves				Mature leaves
	Number of plants	Number of leaves	Total damage (%)	Pathogen damage (%)	Total damage
Inga acuminata	153	727	23.3	1.6	0.11%
Inga cocleensis	319	1461	23.6	3.6	0.30%
Inga goldmanii	149	544	22.8	1.0	0.12%
Inga laurina	91	362	31.4	0.5	0.22%
Inga marginata	131	596	40.6	1.1	0.17%
Inga nobilis[1]	128	559	24.3	4.5	0.19%
Inga pezizifera	223	958	25.4	2.3	0.31%
Inga sapindoides	167	579	22.4	1.2	0.46%
Inga umbellifera	192	843	20.5	4.0	0.23%
Inga vera	86	1672	26.8	6.4	na
Average for *Inga*	1639	8301	26.1	2.6	0.23%
32 other species[2]	1299	47,610	24.9	4.2	na

Data for expanding leaves are for ten species of *Inga* and 32 shade-tolerant tree taxa on Barro Colorado Island, Panama. Saplings were visited monthly during both wet and dry seasons, and the percent of leaf area lost was quantified for all leaves that had flushed and finished expanding during the previous month. Data on expanding leaves were taken from February 2000 to November 2004 for *Inga*, and from 1995 to 2000 for the other 32 species. The percent leaf area lost for mature *Inga* leaves (right column) was measured over 120 days on ~ 50 plants per species and 2–3 leaves per plant. Values are the total percent of leaf area lost during a 31-day period to make an equivalent comparison to the values for young leaf damage

[1] Previously referred to as *Inga quaternata*

[2] *Alseis blackiana* (Rubiaceae), *Capparis frondosa* (Capparaceae), *Chrysophyllum panamense* (Sapotaceae), *Connarus turczaninowii* (Connaraceae), *Cupania rufescens* (Sapindaceae), *Cupania sylvatica* (Sapindaceae), *Desmopsis panamensis* (Annonaceae), *Eugenia oerstediana* (Myrtaceae), *Garcinia madruno* (Clusiacae), *Guatteria dumentorum* (Annonaceae), *Gustavia superba* (Lecythidaceae), *Heisteria concinna* (Olacaceae), *Hirtella triandra* (Chrysobalanaceae), *Hybanthus prunifolius* (Violaceae), *Laetia thamnia* (Salicaceae), *Licania platypus* (Chrysobalanaceae), *Mouriri parvifolia* (Melastomataceae), *Myrcia fosterii* (Myrtaceae), *Ouratea lucens* (Ochnaceae), *Paulinia bracteosa* (Sapindaceae), *Paulinia rugosa* (Sapindaceae), *Piper cordulatum* (Piperaceae), *Poulsenia armata* (Moraceae), *Prioria copaifera* (Leguminosae), *Psychotria horizontalis* (Rubiaceae), *Psychotria limonensis* (Rubiaceae), *Psychotria marginata* (Rubiaceae), *Rourea glabra* (Connaraceae), *Sorocea affinis* (Moraceae), *Talisia princeps* (Sapindaceae), *Tetragastris panamensis* (Burseraceae), *Trichilia tuberculata* (Meliaceae)

Table 2 A latitudinal survey of daily rates of predation for clay caterpillars in the understory of broad-leaved forests

Location	%/day	N	Latitude	Elev (m)	Dates	Sites
Utah, USA	2.0	1898	40.8	1555	May–July 2000	Red Butte, City Creek
Connecticut, USA	7.5	320	37.1	12	Sept 2005	Wadsworth Falls
Panama	13.6	441	9.2	400	Oct–Dec 1999, 2005	Barro Colorado Island
Peru	72.7	712	12.3	270	Jun–Nov 2007	Los Amigos Biological Station
Brazil	35.0	240	2.4	260	Jul–Sept 2005	Km 41, PDBFF
Central African Republic	45.0	289	2.2	360	Oct–Nov 2004	Mondika Research Center
Uganda	29.1	402	0.3	1100	Sept 2004	Kibale National Park

Caterpillars (2 × 20 mm) made out of green Sculpey II clay were glued with a dab of rubber cement to the midrib of mature leaves and censused 24 h later for predation marks (Richards and Coley 2007). *N* is the number of caterpillars. Latitude, elevation, sampling dates and specific site locations are indicated. Panama experiences a more severe dry season than other tropical sites, and Uganda is at a higher elevation

Defenses of *Inga*

Most plants have a variety of defenses that act in concert to successfully ward off nearly all potential herbivores. There is no magic bullet, so it is the entire defensive profile that determines susceptibility to the community of herbivores. Thus, we have quantified as much of the defensive arsenal as possible (Fig. 2) to understand tradeoffs, effectiveness and evolutionary relationships among defensive traits. Here, we present data on the defenses of expanding leaves,
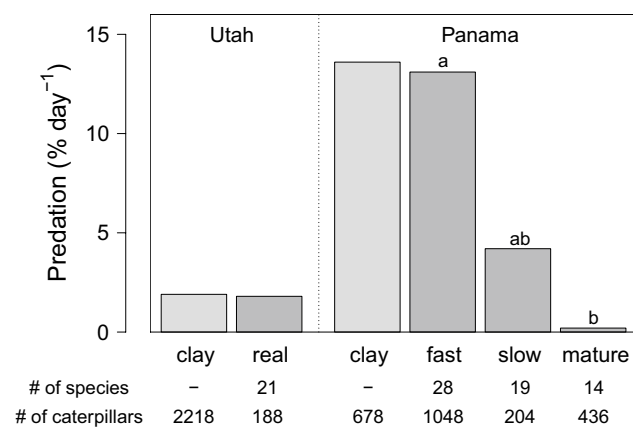
Fig. 1 Daily predation rates on clay and real caterpillars for Utah, USA, and Barro Colorado Island, Panama. Real caterpillars in Panama were divided into three functional groups based on whether they specialized on fast-expanding, slow-expanding or mature leaves. Real caterpillars with different letters are significant at $P < 0.05$

as that is the period of greatest attack by herbivores. We have also focused on saplings, as they often persist in the understory for decades, and are a key bottleneck in the life-cycle of tropical trees (Poorter 2007; Green et al. 2014).

Secondary metabolites

In *Inga* leaves, soluble secondary metabolites are a key defense (Endara et al. 2017). Recently, we have used untargeted metabolomics to characterize all soluble compounds with intermediate polarity, primarily phenolics and saponins (Endara et al. 2015; Wiggins et al. 2016). Regarding highly polar, soluble secondary metabolites, *Inga* species make non-protein amino acids from at least three different amino acids (lysine, proline and cysteine). One clade with 17 species overexpresses tyrosine, an essential amino acid that is highly toxic at 5–19% of dry weight (DW) found in expanding leaves (Lokvam et al. 2006). Expanding leaves invest substantial resources in these soluble secondary metabolites, averaging 45% DW (Lokvam and Kursar 2005; Wiggins et al. 2016).

We have bioassayed the chemical fractions in *Inga* with a generalist caterpillar (*Heliothis virescens*; Coley et al. 2005; Lokvam and Kursar 2005; Lokvam et al. 2006; Brenes-Arguedas et al. 2008; Bixenmann et al. 2016; and unpublished). We found high toxicity of the phenolic and saponin fractions, and of tyrosine, even at concentrations considerably lower than what are found naturally in expanding leaves. The non-protein amino acid fraction showed low toxicity. Nevertheless, some that we did not test such as djenkolic acid, a cysteine derivative that is abundant in *Inga acuminata* may be highly toxic (Segasothy et al. 1995). The protein, organic acid/carbohydrate and lipid fractions were non-toxic. This suggests that several distinct classes of compounds found in *Inga* (phenolics, saponins, non-protein amino acids and tyrosine) have a defensive role. An additional 20% of DW is covalently bound to cell walls, and bioassays with *H. virescens* show that this fraction is also highly toxic (Lokvam and Kursar 2005). Most studies ignore cell wall-bound compounds, yet this fraction contains a
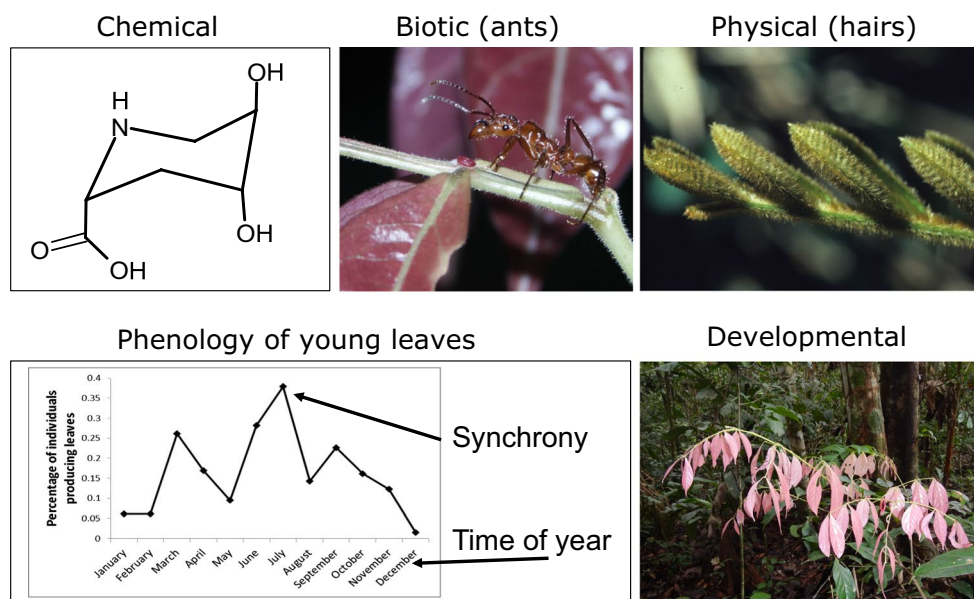

Fig. 2 Six independent classes of defenses for expanding *Inga* leaves. Only trichomes (length and density) showed a phylogenetic signal. Because leaves cannot toughen their cell walls while expanding, they remain tender. Therefore, toughness, which may be the most effective defense, is only an option for mature (fully expanded leaves)

substantial investment in toxic metabolites. Thus, including soluble and cell wall-bound metabolites, over half of the dry weight of an expanding leaf is invested in chemical defenses.

Little induction in secondary metabolites

Theoretical studies suggest that constitutive defenses will be favored if herbivore pressure is constantly high and that induction will be advantageous if herbivory is variable and current damage is a reliable predictor of continued attack (Adler and Karban 1994). Although many studies have shown that species can induce defenses, we argue that this strategy does not make sense for expanding leaves in the tropics where herbivore pressure is constantly high. Indeed, a study of mature leaves of 17 rainforest tree species showed no induction (Cárdenas et al. 2015). In the case of *Inga,* expanding leaves also show little or no quantitative or qualitative induction of metabolites in response to herbivory (Bixenmann et al. 2016). In experiments with four species of *Inga* in Panama, we placed generalist caterpillars from the family Gelechiidae on expanding leaves and kept other plants herbivore-free. Despite the fact that 5–66% of leaf area was eaten in the herbivory treatments, there was no difference in the total investment in saponins, and only a 13% increase in phenolics. Comparing herbivory plus and minus treatments, there was no differential expression of individual compounds (saponins and phenolics), so their metabolic profiles were similar. The major effect of herbivory was a doubling of tyrosine in *I. umbellifera*. Herbivory also did not affect the production of nectar at extrafloral nectaries (Bixenmann et al. 2011). This is not because plants are incapable of adjusting, as both light and the presence of ants increased nectar production. In addition, *Inga* leaves are expanding for only 1–3 weeks and, in Panama, lose 20–45% of their leaf area (Kursar et al. 2006). Under such high herbivore pressure, even a delay of 1 day to induce defenses could result in substantial damage. In addition, given that more than 50% of DW is in constitutive soluble and cell wall-bound secondary metabolites (Lokvam and Kursar 2005; Wiggins et al. 2016), it might be physiologically impossible to increase levels substantially.

Investment in chemical defense in expanding leaves is highly canalized, exhibiting little plasticity, not only with respect to herbivory, but also to light. Because of increased carbon acquisition at higher light, carbon-based defenses such as phenolics are expected to increase (Bryant et al. 1983), with typical increases in mature leaves of tropical species of 200% or more (Sinimbu et al. 2012, Supplement 3). However, for expanding leaves of four *Inga* species from Panama in understory vs. light gap conditions, there was no difference in their metabolomic profiles (Bixenmann et al. 2016). There was also no increase in phenolic content for any species and, for two species, only a 6 and 9% increase in saponins. A Brazilian *Inga* showed no difference in saponins

in shade vs. gap conditions, and a 20% increase in phenolics in gaps (Sinimbu et al. 2012). Thus, even though mature leaves generally respond strongly to light, we found little effect of light on expanding leaves.

Extrafloral nectaries

Inga is also protected by extrafloral nectaries situated between each pair of leaflets. When the leaf is expanding, they produce sugar which attracts protective ants and reduces herbivory (Koptur 1984). Nectar production (sugar/nectary/day) and nectary size do not change during leaf expansion. That is, at only 1% of full leaf size, nectar production and nectary size are already at their maxima. This means that early in leaf expansion, nectar per leaf area, and hence the density of patrolling ants is high (Bixenmann et al. 2013). Investment in nectar per leaf area declines during expansion, and nectar production ceases entirely at full size and leaf toughening.

Inga species vary considerably in investment in nectar production, which influences the number of ants visiting leaves (Bixenmann et al. 2011). For seven species in Panama, average nectar production ranged from 0 to 277 μg sugar/nectary/day and was composed of sucrose, fructose and glucose. We have not quantified nectar components such as amino acids. For both natural nectar and artificial sugar water, ant visitation increased significantly with elevated levels of nectar. For 95 species of *Inga* in Central and South America nectary size ranged from 0.3 to 4.5 mm (Fig. S2) and species with larger nectaries had more ant visitors (Fig. 3). The 35 species of *Inga* at a single site in Peru also differed in the community composition of ants at nectaries (Endara et al. 2017). Some of this is due to differences in the ant communities in terre firme vs. seasonally flooded forest, with the remainder presumably due to interspecific variation in rates of nectar production, and possibly composition.

Trichomes

As with many tropical species, some *Inga* species have non-glandular hairs on the leaf surface and veins (Pennington 1997). These can provide a physical defense, particularly against early instar caterpillars (Agrawal 1999). The spacing and length of hairs can also influence which ant species patrol the leaf, perhaps allowing the plant to favor more beneficial ants (Davidson et al. 1989).

Rates of leaf expansion

As expanding leaves are preferred, shortening this window of vulnerability should reduce herbivory. However, within *Inga* (Fig. 4), and across > 175 tropical species on all continents (Coley and Kursar 1996), species with faster
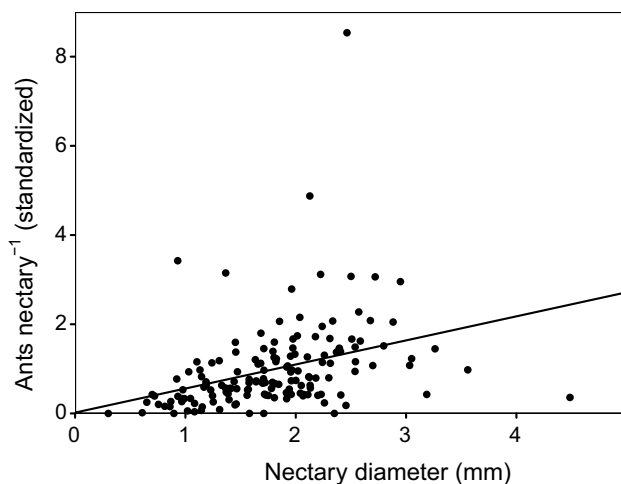
Fig. 3 Mean extrafloral nectary diameter and the standardized number of ants visiting each nectary at five sites in Central and South America (Panama, Ecuador, Peru, Brazil and French Guiana). There were 155 species-site combinations. Because the abundance of ants is different at different sites (low in Panama, high in Peru), we standardized across sites by dividing the number of ant visitors per species by the average visitation at that site ($P < 0.001$, $R^2 = 0.12$)
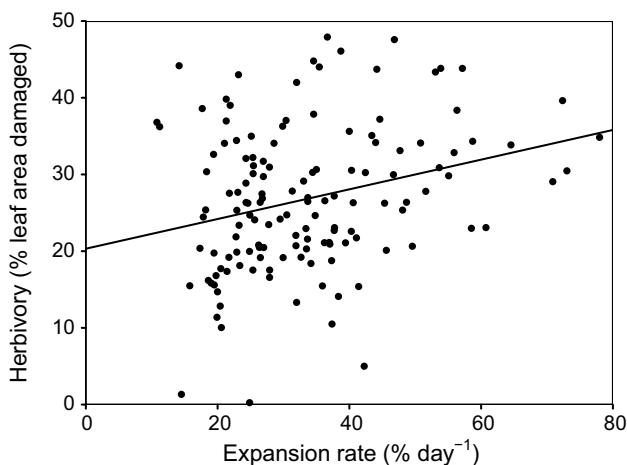

Fig. 4 Rates of leaf expansion (%/day) are correlated with the percent of leaf area lost during expansion at five sites in Central and South America (Panama, Ecuador, Peru, Brazil and French Guiana; $P = 0.001$, $R^2 = 0.08$, $N = 155$ species-site combinations)

expansion had higher total herbivore damage during leaf expansion. Most likely, physiological constraints cause rapid expansion to correlate with traits that promote herbivory. For example, expansion is positively correlated with nitrogen content, as fast expanders, which can double in size daily, divert resources to growth and require high levels of protein (Kursar and Coley 1991, 2003). Thus, fast-expanding species are more nutritious. Additionally, fast expanders appear to have less harmful secondary metabolites. In pairs of non-*Inga* from Panama, extracts from fast expanders were less

toxic to caterpillars, beetles and fungi (Kursar and Coley 2003).

Synchrony and timing of leaf production

The phenology of young leaf production may in part be selected by herbivore pressure. In the tropics, leaf production is less constrained by abiotic factors than in the temperate zone, and yet we still see periods of synchronous leaf flushing (Coley and Aide 1991). Some of this can be attributed to more sun at the beginning of the dry season, and greater soil moisture at the beginning of the rainy season, leading to bursts of production at these times (van Schaik et al. 1993; Angulo-Sandoval and Aide 2000). But even in aseasonal habitats, synchrony is pronounced, as it can provide escape from herbivory by satiating available herbivores (Aide 1988; Léotard et al. 2008; Lamarre et al. 2014). The time of year that leaves are produced can also influence herbivory (Wolda 1988; Aide 1993). For example, there are fewer herbivores in the dry season, and many herbivores are univoltine or seasonal. Hence, for expanding leaves, both the synchrony and timing of leaf production can be defenses.

Constraints and tradeoffs in the evolution of defenses

Because resources are limited, allocation tradeoffs are common. This has led to the identification of syndromes of co-occurring traits. As mentioned above, rapid leaf expansion is correlated with higher herbivory, suggesting that fast expanders may have less effective defenses. The advantages of effective chemical defense are obvious, so we interpret the strategy of rapid expansion as being a sub-optimal strategy. One evolutionary scenario is that if a species' secondary metabolites have been evolutionarily breached by herbivores, selection would favor shortening the window of vulnerability (Kursar and Coley 2003). So, rapid expansion may not be an effective defense, but instead may be a way of minimizing the negative impacts of herbivory by reallocating resources from ineffective chemical defense to growth.

Expansion rate is also highly negatively correlated with chlorophyll content in *Inga* (Fig. 5) and hundreds of other tropical species (Coley and Kursar 1996; Kursar and Coley 2003). Chlorophyll content is indicative of chloroplast development, and fast expanders delay chloroplast development until the leaves are full size and toughened (Kursar and Coley 1992a, b, c). As a consequence, they appear white or very light green and have much lower photosynthetic rates. This negative correlation could be driven by a physiological constraint prohibiting simultaneous investment in rapid expansion and chloroplast development.

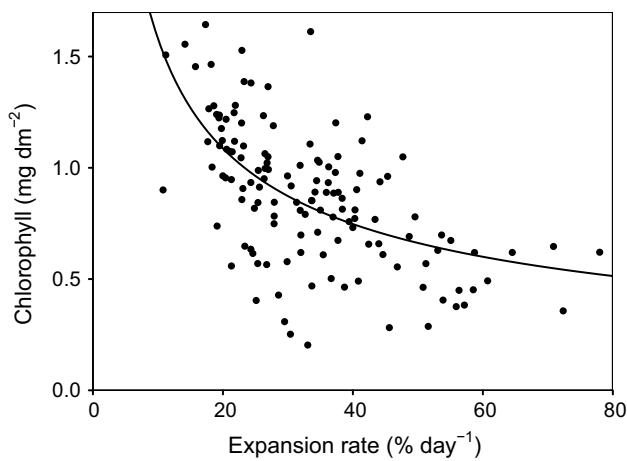However, delayed greening could also be adaptive by minimizing the negative impact of herbivory (Kursar and

Fig. 5 Rates of leaf expansion (%/day) are negatively correlated with chlorophyll content (mg of chlorophyll dm^{-2} of leaf) of expanding leaves at five sites in Central and South America (Panama, Ecuador, Peru, Brazil and French Guiana; $P < 0.001$, $R^2 = 0.39$, $N = 138$ species-site combinations). Chlorophyll was measured when leaves were 50–80% of full size (Kursar and Coley 2003; Kursar et al. 2009)

Fig. 6 Rates of leaf expansion (%/day) are correlated with the degree of synchrony among individual plants within ten *Inga* species flushing young leaves on Barro Colorado Island, Panama. From March 2001 to November 2004, 50 individuals of each species were censused monthly for leaf production for 5 years. A higher value indicates more synchronous production of leaves ($P = 0.03$, $R^2 = 0.46$)

Coley 1992c). For a given amount of damage, leaves with delayed greening lose ~ 20% fewer resources as they have not yet invested in chloroplasts. Thus, under conditions of low light (< 1% full sun) and high herbivory (> 30% leaf area lost), the costs of forfeited photosynthesis match the benefits of reduced losses. This set of conditions is only commonly found in the understory of tropical rainforests and is the only environment that favors white young leaves. Furthermore, the cost/benefit analysis predicts that delayed greening should be more common in fast-expanding, shade species as they suffer high losses to herbivores. As with other traits, delayed greening is developmentally canalized, such that even if saplings are in the high light of a treefall gap, they still delay greening (Kursar and Coley 1992a).

We also suggest that species with less effective chemical defenses may have more synchronous leaf production, even in aseasonal environments, due to selection by herbivores. Given that there is an opportunity cost to synchrony associated with storing and defending resources, species with young leaves having effective chemical defenses should produce a leaf as soon as there are enough resources (a lack of synchrony). In Panama, we tracked leaf production by saplings for 4 years and calculated synchrony as the CV in the number of individuals leafing each month. There was a positive relationship between expansion rate and synchrony for 25 species (Coley and Kursar 1996, $R^2 = 0.36$, $P < 0.001$) and for 10 species of *Inga* (Fig. 6), consistent with a syndrome of high synchrony in species with weaker chemical defenses (Kursar and Coley 2003).
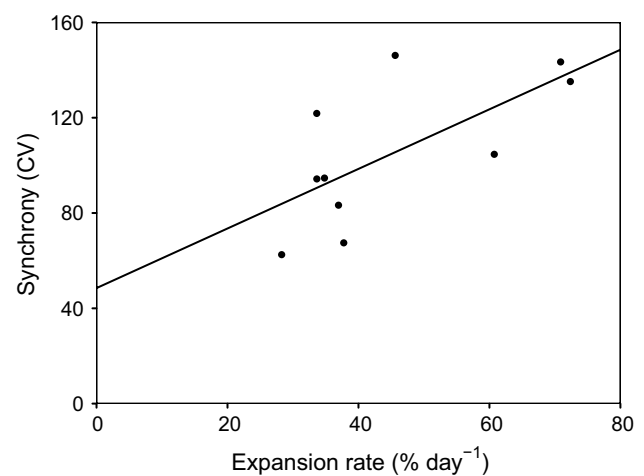
Divergent selection on defenses

Despite physiological or genetic constraints that may promote trait correlations, we find that many traits evolve independently, providing further evidence that herbivores may be driving selection. For 33 *Inga* species from Peru, the six classes of defense (Fig. 2) are orthogonal in trait space (Endara et al. 2017), indicating that each defensive category has evolved independently of the others. The trichome axis represents both length and density of trichomes, which were correlated. Similarly, the ant-defense axis includes the correlated number and identity of visiting ants, and the two correlated developmental traits are expansion rate and chlorophyll. Although we treat secondary metabolites as a single class, there are hundreds of compounds that can vary independently. Thus, there are many axes of trait divergence that are possible, leading to an infinite number of trait combinations (Coley and Kursar 2014).

The six different classes of *Inga* defenses (Fig. 2) are not only evolving independently, but are also diverging between close relatives (Kursar et al. 2009; Endara et al. 2017). Only trichomes show a phylogenetic signal, suggesting that the evolution of defensive traits is evolutionarily labile. Divergence is also documented for chemical defenses in other speciose tropical genera, *Bursera, Psychotria, Protium, Solanum* and *Piper* (Becerra 1997; Sedio 2013; Fine et al. 2013a, b; Haak et al. 2014; Salazar et al. 2016a). In contrast, although we measured very few traits associated with resource acquisition, these appear to evolve more slowly (Endara et al. 2015). A similar pattern has been found for

Psychotria and *Solanum* (Sedio et al. 2012; Haak et al. 2014). Thus, it appears that selection from herbivores causes strong divergent selection for defensive traits (Marquis et al. 2016). Nevertheless, much more work is needed to tease apart the relative importance of divergence in defenses vs. resource acquisition traits in the evolution of new species.

Defenses shape local tree diversity

The diversity of trees in a single hectare of tropical rainforest (655 species) exceeds that of the entire US and Canada combined (less than 500 species). This contrast requires explanation, particularly for speciose genera such as *Inga*, where 45 closely related species can coexist at a single site (Valencia et al. 2004). The high local diversity is generally thought to result from negative density dependence, such that no one species 'wins'. One possible driver of negative density dependence is that competition for resources will be greater for conspecifics (Chesson 2000). However, since all trees need similar resources (light, water and nutrients), it is difficult to see how there could be 655 different niches in a single hectare (Wright 2002; Sedio et al. 2012). An alternative explanation for negative density dependence is that specialized pests keep one species from dominating (Janzen 1970; Connell 1971; Kursar et al. 2009; Terborgh 2012; Bagchi et al. 2014). And, if one considers the almost infinite number of possible defense profiles, there could be an enormous number of niches with respect to herbivores (Singer and Stireman 2005; Kursar et al. 2009; Coley and Kursar 2014). We hypothesize that if neighbors differ in defense, they are unlikely to share herbivores and coexistence is enhanced. Most examples of negative density dependence show that conspecifics, which would share traits, are over-dispersed (Harms et al. 2000; Comita et al. 2014; Kraft et al. 2015a, b; Zhu et al. 2015; LaManna et al. 2017). But this does not identify the responsible mechanisms, competition for resources or pest pressure, nor the key traits, defenses or resource-acquisition traits.

Our work on *Inga* is one of the few studies to measure defensive traits and test their effect on promoting local diversity and coexistence. For *Inga* in both Panama and Peru, neighboring saplings were more different in their defenses than would be expected by a random draw of species in that forest (Kursar et al. 2009). This was true even for Peru, where neighbors were more closely related than expected by chance. This pattern is consistent with selection by herbivores, favoring rare defenses whether or not species are closely related (Becerra 2007; Coley and Kursar 2014; Richards et al. 2015; Salazar et al. 2016a, b).

We suggest that the composition of *Inga* species in a given forest may also be shaped by herbivores. *Inga* lineages have dispersed across the Amazon Basin repeatedly, such that the species pool at any site is potentially drawn from across the Amazon (Dexter et al. 2017). What determines which species actually occur at a site no doubt includes abiotic filters, but perhaps also the defensive profiles of the co-occurring species. We predict that the species at a site will be more different in defenses than expected from a random draw of all Amazonian species. Thus, at spatial scales of meters to kilometers, herbivores may influence host community composition.

Chemistry: evolution of innovations and divergence between species

One long-standing prediction from coevolutionary theory is that key innovations in host clades, such as glucosinolates or latex, lead to radiations in hosts and then in herbivores (Ehrlich and Raven 1964; Farrell et al. 1991). It is generally assumed that the evolution of these novel secondary metabolites is through changes in the genes that code for biosynthetic enzymes (Berenbaum 1978, 1981; Ryan et al. 2012). However, such biosynthetic innovations are relatively rare, and hence their signature is typically seen at the level of family or genus. We hypothesize that chemical dissimilarity between species allows plants to escape from herbivory, regardless of whether they are based on combining common metabolites or from biosynthetic innovations.

Chemical dissimilarities based on compounds composed of common building blocks

The genus *Inga* does not manifest any compounds that are based on biosynthetic innovation. Instead, an important source of interspecific variation in chemistry is due to different, and sometimes novel, compounds composed of common building blocks (Kursar et al. 2009). Examples in *Inga* are compounds based on only (epi)catechin, glucose and cinnamic acid that were new to science (Lokvam et al. 2004; Fig. 7, compound #27 without gallate). Evolution of these compounds in *Inga* may proceed by changes in the regulation of genes that code for their synthesis or the genes that code for condensation enzymes such as acyltransferases (Barrier et al. 2001; Wray 2007). For example, for a flavan-3-ol, such as compound #27 (Fig. 7), this would be through upregulation of the synthesis of (epi)catechin, gallate, glucose and cinnamate, and variations in acylation, glycosylation, and galloyltransferase activities. Because most condensing enzymes belong to large gene families, such evolutionary transitions could be due to gene duplication followed by the evolution of novel substrate affinity, without the evolution of a unique enzymatic reaction (Bontpart et al. 2015).

Chemical dissimilarities based on novel combinations of compounds

The most common pattern of chemical divergence in *Inga* is based on each species having different combinations of all the compounds that *Inga* can make. For example (Fig. S4), one *Inga* species contains compound #30 (Fig. 7, (epi)gallocatechin gallate) and compound #33 (a triterpene saponin, Fig. S3), whereas another species also contains compound #30 but combined with flavone glycosides (FG, Fig. S4). Thus, closely related species of *Inga* diverge via novel combinations of compounds leading to dissimilar chemical profiles rather than via the evolution of key biosynthetic innovations. Similar patterns may exist in other genera (Lokvam et al. 2015). A likely mechanism for creating new combination of compounds may be through gene regulation.

Evidence for evolution by changes in gene regulation

For phenolic compounds, the most abundant of the toxic compounds in *Inga*, the known biosynthetic pathways provide insight into the underlying genetic mechanisms of metabolite evolution (Fig. 7). When mapping compounds onto the phylogenetic tree for Peru and Panama, a particular metabolite class (e.g. quinic acid gallates, (epi)catechin polymers, (epi)gallocatechin gallate polymers, saponins, tyrosine gallates) occurs sporadically across the phylogenetic tree (Kursar et al. 2009). In fact, in some cases, expression is in only one member of a pair of closely related species. It is unlikely that the ability to synthesize each compound evolved independently many times. Instead, the most parsimonious explanation is that all species have the genes coding for the biosynthetic enzymes for each compound, but these are turned on or off in different species. We also often detect trace amounts of common compounds by ultra-performance liquid chromatography followed by mass spectrometry (UPLC–MS), supporting the hypothesis that structural genes are present but strongly downregulated.

Another example consistent with gene regulation is that in 5 of the 42 species from Panama and Peru, 80% or more of the secondary metabolites are triterpene saponins, with the remainder being phenolic compounds (Kursar et al. 2009; unpublished). As the shikimic acid pathway is required for primary metabolism, and as the five species are spread throughout the phylogeny, with close relatives making phenolics, the production of saponins and the repression of phenolics most likely result from regulatory changes (Mertens et al. 2016). Additionally, the tyrosine-accumulating clade mentioned previously (Fig. 7: compound #4) is most simply explained as a change in regulation without a change in biosynthesis. Another example is the intriguing pattern that

species make adducts or derivatives of either compound #14 or of compound #18 but not both, suggesting an important switchpoint at compound #10 (Fig. 7). Furthermore, each chemotype is spread across the phylogeny and close relatives may differ. It appears that, during evolution, this switchpoint has repeatedly and reversibly converted between two states.
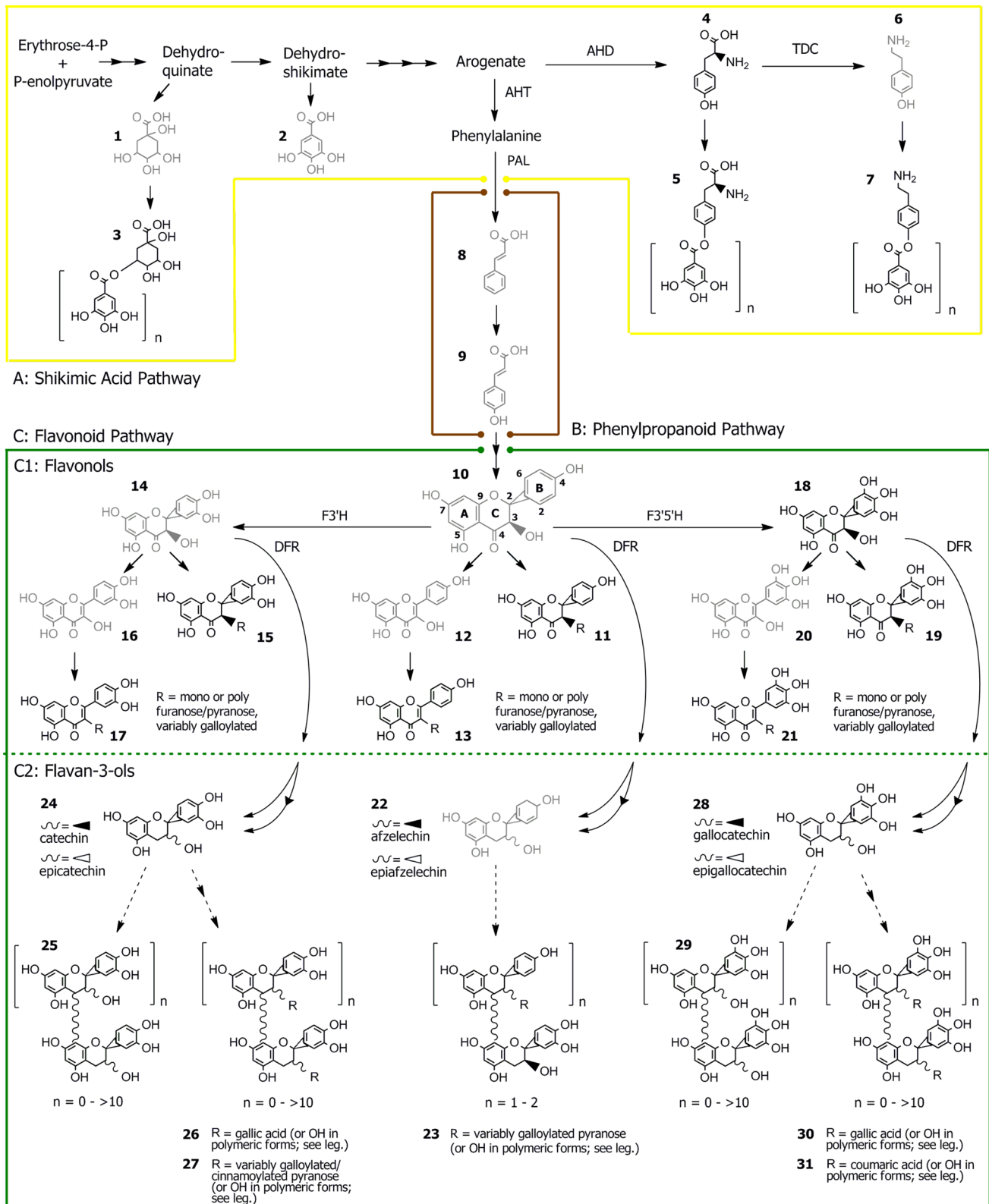
Evolutionary patterns

If secondary metabolism evolves in *Inga* by the simple mechanisms suggested above, this may permit rapid evolution and explain why closely related species are chemically distinct (Kursar et al. 2009; Endara et al. 2017). Large and fast shifts in defensive chemicals, such as between saponins and phenolics, also may permit evolution in long-lived trees to keep pace with rapid evolution (short generation times and large population sizes) of most insect herbivores (Gould 1991).

A critical deficiency in the study of the evolution of secondary metabolites is that most compounds are not identified structurally. As a result, while one can quantify compounds that are shared by species, the analysis of non-shared compounds is highly problematic unless one can distinguish structurally related from unrelated compounds. Structural similarity is important because it may indicate similar functions with respect to anti-herbivore activity, as well as similar biosynthetic pathways. These both shed light on the evolution of secondary metabolites and their effectiveness as defenses. One approach to quantify structural similarity that we use is UPLC followed by isolation of the molecule of interest using MS, its fragmentation, and the detection of fragments (MS–MS). MS–MS provides a structural fingerprint such that the relatedness of compounds can be quantified using networks (Duncan et al. 2015; Sedio 2017; Sedio et al. 2017). In addition, we use UPLC–MS to detect many more compounds than in HPLC. At present, the combination of UPLC–MS with MS–MS may be one of the most rigorous approaches for quantifying the chemical similarity of species (but see Richards et al. 2015).

Evolutionary responses of insect herbivores to plant defenses

High degree of specialization

The lepidopteran herbivores attacking *Inga* are predominantly highly specialized, with most species restricted to three or fewer hosts, and only a few being more generalized (Fig. S5; Dyer et al. 2007; Forister et al. 2015). Based on our data from Panama, French Guiana, Ecuador and Peru, 60% of 483 caterpillar species are associated with a single

A: Shikimic Acid Pathway

B: Phenylpropanoid Pathway

C: Flavonoid Pathway

C1: Flavonols

C2: Flavan-3-ols

host species. Given that different herbivore clades have distinct physiologies and that different plant species may host distinct communities of specialist herbivores (Endara et al.

2017), we hypothesize that each herbivore community exerts dissimilar selective pressure on their hosts. In short, high specificity in herbivores could generate divergent evolution

◀ **Fig. 7** Biosynthetic context of phenolic metabolites in *Inga*. Shown here are the structures and substructures of compounds observed in a survey of 38 phenolic-synthesizing *Inga* species and their positions in the biosynthetic pathways that produce them. Metabolites that accumulate are shown in black; adducts and intermediates in gray. Wavy bonds indicate variable stereochemistry. Shikimic acid pathway: **1** quinic acid; **2** gallic acid; **3** quinic acid gallate; **4** L-tyrosine; **5** tyrosine gallate; **6** tyramine; **7** tyramine gallate. Phenylpropanoid pathway: **8** cinnamic acid; **9** coumaric acid. Flavonoid pathway: **10** dihydrokaempferol (commonly used flavonoid numbering scheme is shown); **11** dihydrokaempferol glycoside; **12** kaempferol; **13** kaempferol glycoside; **14** dihydroquercetin; **15** dihydroquercetin glycoside; **16** quercetin; **17** quercetin glycoside; **18** dihydromyricetin; **19** dihydromyricetin glycoside; **20** myricetin; **21** myricetin glycoside; **22** (epi)afzelechin; **23** catechin-(epi)afzelechin mixed polymers; **24** (epi)catechin; **25** (epi)catechin polymer; **26** (epi)catechin-3-*O*-gallate; **27** (epi)catechin-3-*O*-pyrano-*O*-gallate/*O*-cinnamate; **28** (epi)gallocatechin; **29** (epi)gallocatechin polymer; **30** (epi)gallocatechin-3-*O*-gallate; **31** (epi)gallocatechin-3-*O*-coumarate. For the polymeric forms of **23**, **26**, **27**, **30**, and **31**, the C-3-*O* position is variably substituted with the indicated groups or with OH, resulting in polymers consisting of mixed monomeric units. Key enzymatic steps (solid arrows) are labeled: *AHD* arogenate dehydrogenase, *TDC* tyrosine decarboxylase, *AHT* arogenate dehydratase, *PAL* phenylalanine-ammonia lyase, *F3'H* flavonoid-3'-hydroxylase, *F3'5'H* flavonoid-3',5'-hydroxylase, *DFR* dihydroflavone reductase. Dashed arrows represent biosynthetic steps of unknown enzymology

of defenses in *Inga* and this mechanism could drive ecological speciation (sensu Nosil 2012), and adaptive radiation (sensu Schluter 2000). Two other groups sawflies (Hymenoptera) and Coreidae (Hemiptera) that cause substantial herbivory show distinct patterns of specialization. Sawflies are highly specialized, feeding on one or two hosts, whereas Coreidae feed on most, and possibly all, *Inga* at a site.

Herbivores chose hosts based on traits

Most studies on host range for insect herbivores consider host phylogeny but often do not consider anti-herbivore traits. Although the significance of plant defenses in host selection is recognized, this has been insufficiently studied for insect herbivores of the tropics. Traditionally, secondary plant chemistry has been invoked as a major axis in host selection (Thompson 1988), and our studies with *Inga* and its associated herbivores are consistent with this view. For example, a choice experiment with a sawfly larva showed that even small differences in secondary metabolites among its normal hosts within the *I. capitata* complex were big enough to affect preference (Endara et al. 2015). In Peru, 30% of the variation in the herbivore community was explained solely by chemistry, and it was consistently identified as an important variable across specific lepidopteran clades (Endara et al. 2017). Larvae from the Gelechioidea and Riodinidae show preference for *Inga* hosts that express saponins, whereas Erebidae are more abundant on plants that express amines. These results are of particular interest

because it suggests that specific classes of secondary metabolites strongly influence host choice (Berenbaum 1981).

Nevertheless, as was noted, in addition to chemistry, plants invest in a variety of important defenses (Carmona et al. 2011). Our analyses show that different clades of Lepidoptera respond to different non-chemical defenses of *Inga* (Endara et al. 2017). For example, for Riodinidae, a group of myrmecophilous larvae, ant associations with *Inga* explained as much as 30% of their total community variation (Endara et al. 2017). By contrast, for Lycaenidae, a sister family of Riodinidae, ants did not represent a significant factor for host selection. This pattern is expected given that Lycaenidae feeding on *Inga* are not tended by ants. This result highlights the fact that, even for sister clades of herbivores, differences in plant defenses matter for host use.

The fact that the expression of defenses in *Inga* is independent from its phylogeny (discussed above) has allowed us to show that host switching in lepidopteran herbivores is more strongly associated with host defenses than host phylogeny (Endara et al. 2017). Thus, closely related herbivores prefer *Inga* hosts with similar defenses rather than closely related *Inga*. Similar patterns have been found for other groups of herbivores, including leaf beetles (*Blepharida*), several groups of herbivores associated with Apiaceae, and larvae from Melitaeini species (Becerra 1997; Berenbaum 2001; Wahlberg 2001). These results are consistent with the hypothesis that herbivore lineages have evolved new host associations with plant species for which they are preadapted (Agosta and Klemens 2008).

Herbivore diversification

Although it is certain that plant defenses have shaped the evolution of the herbivore traits associated with host use, what promotes herbivore diversification is less clear. The fact that *Inga* herbivores show phylogenetic conservatism in host use suggests that the traits of the herbivores that determine host choice are evolving relatively slowly such that related herbivores feed on plants with similar defenses. We hypothesize that the diversification of the Lepidoptera associated with *Inga* is not solely driven by their interactions with host defenses. Other factors, such as predation, can influence host choice and, possibly, diversification (Singer and Stireman 2005). In *Inga*, we find that the risk of predation is related to host plant traits, implicating a linkage between top-down and bottom-up forces. Nutritional and defensive properties of leaves determine herbivore growth rates, with slower growth increasing the susceptibility to predation. Because caterpillars that feed on mature leaves grow slowly, taking about 1 month to develop, predation rates of only 5% per day or greater would not allow enough time for most larvae to complete development. Consequently, mature leaf feeders have been selected for defenses, and

show significantly more hairs, spines, warning coloration and gregarious behavior than herbivores feeding on expanding leaves (Coley et al. 2006). Due to effective defense, they suffer low rates of actual predation in the field (Fig. 1) or in feeding trials with ants (Coley et al. 2006). In contrast, herbivores that feed on fast-expanding leaves must also grow fast so as to complete development in the ~ 7 days before leaves toughen (Aide and Londoño 1989). The premium on fast growth means caterpillars can invest little in defense and as a consequence suffer high rates of predation in the field (Fig. 1) and in feeding trials with ants. Herbivores that feed on more slowly expanding young leaves have intermediate levels of growth and predation. In summary, interactions with predators may depend on the type of food consumed by herbivores.

Inga and its herbivores: a coevolutionary arms race?

Coevolution has been seen as a major driver of diversification in plants and insects, with diversification for both groups associated with major biosynthetic innovations in chemical defense (Ehrlich and Raven 1964). This predicts phylogenetic constraints in the sense that closely related plant species will have similar defenses and closely related herbivores will feed on closely related plants. Our results with *Inga* favor an alternative hypothesis in which plant defensive traits evolve rapidly and in the absence of biosynthetic innovations. Hence, closely related host plants may not have similar defenses allowing daughter plant lineages to escape a subset of herbivores attacking their parent species.

A second prediction from coevolutionary theory is that trait evolution is stepwise with a shift in defense correlated with a shift in counter-defense (Berenbaum and Schuler 2010), and therefore, evolutionary change between both plants and insects should be reciprocal (Mitter and Brooks 1983). This model is not supported by the result that the phylogenies of insect herbivores rarely match the phylogenies of their hosts. An alternative model is sequential evolution in which the host is colonized by herbivores from closely related host lineages (Futuyma and Agrawal 2009; Janz 2011). In this case, coevolution between herbivore and host would not be a prerequisite for an association, and the diversification of the host usually predates that of the herbivore (Jermy and Szentesi 2003).

However, for *Inga*, comparisons of the ages of diversification of the host with its lepidopteran herbivores and of the topologies of their phylogenetic trees contradict the above models (Endara et al. 2017). First, reciprocity in evolutionary events is not supported in the phylogenetic analyses. We find that unrelated hosts that happen to have similar defenses also support similar herbivore assemblages. Furthermore,

the evolution of host use for *Inga* herbivores is associated more with *Inga* defenses than with *Inga* phylogeny. Second, diversification dates for the associated herbivores predate the diversification of *Inga* (~ 2–10 MY ago, Lavin 2006; Richardson et al. 2001) by many millions of years. For example, 10 of the 15 species of Riodinidae found on *Inga* belong to the genus *Nymphidium*, whose radiation may be at least six times older (50–60 MY ago; Wahlberg et al. 2013; Espeland et al. 2015) than *Inga*. The species of *Nymphidium* found on *Inga* also occur on other legume genera (e.g. *Zygia, Senna* and *Cassia*; DeVries et al. 1992; Janzen and Hallwachs, Caterpillars of ACG database: http://janzen.sas.upenn.edu/index.html). These species may have diverged prior to the *Inga* radiation and switched to *Inga*, or they could have diverged after the *Inga* radiation onto multiple genera of legumes. In either case, we hypothesize that *Nymphidium* shifted onto those hosts for which they had preadaptations.

Thus, our analyses with *Inga* and its herbivores do not support either coevolution or sequential evolution. Instead they are more consistent with macroevolutionary tracking of *Inga* defenses. The interactions between *Inga* and its herbivores appear to be asymmetric. While plants may evolve under selection by herbivores, herbivores may not show coevolutionary adaptations but, instead, may "chase" or track hosts based on host defenses (Brooks and McLennan 2012; Agosta 2006), a non-reciprocal model of host-herbivore trait evolution. This framework suggests that anti-herbivore defenses may evolve more rapidly than the herbivore traits that determine host choice and/or ability to feed and grow successfully, allowing plant species to outpace the relatively short generation times of herbivorous insects.

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References

Adler FR, Karban R (1994) Defended fortresses or moving targets? Another model of inducible defenses inspired by military metaphors. Am Nat 144:813–832. https://doi.org/10.1086/285708

Agosta SJ (2006) On ecological fitting, plant–insect associations, herbivore host shifts, and host plant selection. Oikos 114:556–565. https://doi.org/10.1111/j.2006.0030-1299.15025.x

Agosta SJ, Klemens JA (2008) Ecological fitting by phenotypically flexible genotypes: implications for species associations, community assembly and evolution. Ecol Lett 11:1123–1134. https://doi.org/10.1111/j.1461-0248.2008.01237.x

Agrawal AA (1999) Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. Ecology 80:1713–1723. https://doi.org/10.1890/0012-9658(1999)080[1713:irthiw]2.0.co;2

Aide TM (1988) Herbivory as a selective agent on the timing of leaf production in a tropical understory community. Nature 336:574–575. https://doi.org/10.1038/336574a0

Aide TM (1993) Patterns of leaf development and herbivory in a tropical understory community. Ecology 74:455–466. https://doi.org/10.2307/1939307

Aide TM, Londoño E (1989) The effects of rapid leaf expansion on the growth and survivorship of a lepidopteran herbivore. Oikos 55:66–70. https://doi.org/10.2307/3565873

Angulo-Sandoval P, Aide TM (2000) Leaf phenology and leaf damage of saplings in the Luquillo experimental forest, Puerto Rico. Biotropica 32:415–422. https://doi.org/10.1111/j.1744-7429.2000.tb00488.x

Bagchi R, Gallery RE, Gripenberg S, Gurr SJ, Narayan L, Addis CE, Freckleton RP, Lewis OT (2014) Pathogens and insect herbivores drive rainforest plant diversity and composition. Nature 506:85–88. https://doi.org/10.1038/nature12911

Barone JA (1998) Host-specificity of folivorous insects in a moist tropical forest. J Anim Ecol 67:400–409. https://doi.org/10.1046/j.1365-2656.1998.00197.x

Barrier M, Robichaux RH, Purugganan MD (2001) Accelerated regulatory gene evolution in an adaptive radiation. Proc Natl Acad Sci USA 98:10208–10213. https://doi.org/10.1073/pnas.181257698

Becerra JX (1997) Insects on plants: macroevolutionary chemical trends in host use. Science 276:253–256

Becerra JX (2007) The impact of herbivore–plant coevolution on plant community structure. Proc Natl Acad Sci USA 104:7483–7488. https://doi.org/10.1073/pnas.0608253104

Benrey B, Denno RF (1997) The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. Ecology 78:987–999. https://doi.org/10.1890/0012-9658(1997)078[0987:TSGHMH]

Berenbaum M (1978) Toxicity of a furanocoumarin to armyworms: a case of biosynthetic escape from insect herbivores. Science 201:532–534. https://doi.org/10.1126/science.201.4355.532

Berenbaum M (1981) Patterns of furanocoumarin distribution and insect herbivory in the Umbellifera: plant chemistry and community structure. Ecology 62:1254–1266. https://doi.org/10.2307/1937290

Berenbaum MR (2001) Chemical mediation of coevolution: phylogenetic evidence for Apiaceae and associates. Annu Mo Bot Gard 88:45–59

Berenbaum MR, Schuler MA (2010) Elucidating evolutionary mechanisms in plant-insect interactions: key residues as key innovations. In: Bell MA, Futuyma DJ, Eanes WF, Levinton JS (eds) Evolution since Darwin: the first 150 years. Sinauer Associates, Sunderland, pp 269–290

Bixenmann RJ, Coley PD, Kursar TA (2011) Is extrafloral nectar production induced by herbivores or ants in a tropical facultative ant–plant mutualism? Oecologia 165:417–425. https://doi.org/10.1007/s00442-010-1787-x

Bixenmann RJ, Coley PD, Kursar TA (2013) Developmental changes in direct and indirect defenses in the young leaves of the neotropical tree genus *Inga* (Fabaceae). Biotropica 45:175–184. https://doi.org/10.1111/j.1744-7429.2012.00914.x

Bixenmann RJ, Coley PD, Weinhold A, Kursar TA (2016) High herbivore pressure favors constitutive over induced defense. Ecol Evol 6:6037–6049. https://doi.org/10.1002/ece3.2208

Bontpart T, Cheynier V, Ageorges A, Terrier N (2015) BAHD or SCPL acyltransferase? What a dilemma for acylation in the world of plant phenolic compounds. New Phytol 208:695–707. https://doi.org/10.1111/nph.13498

Brenes-Arguedas T, Horton MW, Coley PD, Lokvam J, Waddell RA, Meizoso-O'Meara BE, Kursar TA (2006) Contrasting mechanisms of secondary metabolite accumulation during leaf development in two tropical tree species with different leaf expansion strategies. Oecologia 149:91–100. https://doi.org/10.1007/s00442-006-0423-2

Brenes-Arguedas T, Coley PD, Kursar TA (2008) Divergence and diversity in the defensive ecology of *Inga* at two neotropical sites. J Ecol 96:127–135. https://doi.org/10.1111/j.1365-2745.2007.01327.x

Brooks DR, McLennan DA (2012) The nature of diversity: an evolutionary voyage of discovery. University of Chicago Press, Chicago

Bryant JP, Chapin III FS, Klein DR (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357–368. https://doi.org/10.2307/3544308

Cárdenas RE, Hättenschwiler S, Valencia R, Argoti A, Dangles O (2015) Plant herbivory responses through changes in leaf quality have no effect on subsequent leaf-litter decomposition in a neotropical rain forest tree community. New Phytol 207:817–829. https://doi.org/10.1111/nph.13368

Carmona D, Lajeunesse MJ, Johnson MT (2011) Plant traits that predict resistance to herbivores. Funct Ecol 25:358–367. https://doi.org/10.1111/j.1365-2435.2010.01794.x

Chesson P (2000) Mechanisms of maintenance of species diversity. Annu Rev Ecol Evol Syst 31:343–366. https://doi.org/10.1146/annurev.ecolsys.31.1.343

Coley PD (1980) Effects of leaf age and plant life history patterns on herbivory. Nature 284:545–546

Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol Monogr 53:209–233

Coley PD, Aide T (1991) Comparison of plant defenses in temperate and tropical broad-leaved forests. In: Price PW, Lewinsohn TM, Fernandes GW, Benson WW (eds) Plant–animal interactions: evolutionary ecology in tropical and temperate regions. Wiley, New York, pp 25–49

Coley PD, Kursar TA (1996) Anti-herbivore defenses of young tropical leaves: physiological constraints and ecological trade-offs. In: Mulkey SS, Chazdon RL, Smith AP (eds) Tropical forest plant ecophysiology. Chapman and Hall, New York, pp 305–336

Coley PD, Kursar TA (2014) On tropical forests and their pests. Science 343:35–36. https://doi.org/10.1126/science.1248110

Coley PD, Lokvam J, Rudolph K, Bromberg K, Sackett TE, Wright L, Brenes-Arguedas T, Dvorett D, Ring S, Clark A (2005) Divergent defensive strategies of young leaves in two species of *Inga*. Ecology 86:2633–2643. https://doi.org/10.1890/04-1283

Coley PD, Bateman ML, Kursar TA (2006) The effects of plant quality on caterpillar growth and defense against natural enemies. Oikos 115:219–228. https://doi.org/10.1111/j.2006.0030-1299.14928.x

Comita LS, Queenborough SA, Murphy SJ, Eck JL, Xu K, Krishnadas M, Beckman N, Zhu Y (2014) Testing predictions of the Janzen–Connell hypothesis: a meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. J Ecol 102:845–856. https://doi.org/10.1111/1365-2745.12232

Connell JH (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: Den Boer PJ, Gradwell GR (eds) Dynamics of populations. PUDOC, Wageningen, pp 298–312

Davidson DW, Snelling RR, Longino JT (1989) Competition among ants for myrmecophytes and the significance of plant trichomes. Biotropica 21:64–73. https://doi.org/10.2307/2388444

DeVries PJ, Chacon IA, Murray D (1992) Toward a better understanding of host use and biodiversity in riodinid butterflies (Lepidoptera). J Res Lepid 31:103–126

Dexter KG, Lavin M, Torke BM, Twyford AD, Kursar TA, Coley PD, Drake C, Hollands R, Pennington RT (2017) Dispersal assembly of rain forest tree communities across the Amazon basin. Proc Natl Acad Sci USA 114:2645–2650. https://doi.org/10.1073/pnas.1613655114

Duncan KR, Crüsemann M, Lechner A, Sarkar A, Li J, Ziemert N, Wang M, Bandeira N, Moore BS, Dorrestein PC, Jensen PR (2015) Molecular networking and pattern-based genome mining improves discovery of biosynthetic gene clusters and their products from *Salinispora* species. Chem Biol 22:460–471. https://doi.org/10.1016/j.chembiol.2015.03.010

Dyer LA, Singer M, Lill J, Stireman J, Gentry G, Marquis RJ, Ricklefs RE, Greeney HF, Wagner DL, Morais H, Diniz IR, Kursar TA, Coley PD (2007) Host specificity of Lepidoptera in tropical and temperate forests. Nature 448:696–699. https://doi.org/10.1038/nature05884

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution 18:586–608. https://doi.org/10.2307/2406212

Endara MJ, Weinhold A, Cox JE, Wiggins NL, Coley PD, Kursar TA (2015) Divergent evolution in antiherbivore defences within species complexes at a single Amazonian site. J Ecol 103:1107–1118. https://doi.org/10.1111/1365-2745.12431

Endara MJ, Coley PD, Ghabash G, Nicholls JA, Dexter KG, Donoso DA, Stone GN, Pennington RT, Kursar TA (2017) Coevolutionary arms race versus host defense chase in a tropical herbivore–plant system. Proc Natl Acad Sci USA 114:E7499–E7505. https://doi.org/10.1073/pnas.1707727114

Espeland M, Hall JP, DeVries PJ, Lees DC, Cornwall M, Hsu Y-F, Wu L-W, Campbell DL, Talavera G, Vila R, Salzman S, Ruehr S, Lohman D, Pierce NE (2015) Ancient neotropical origin and recent recolonization: phylogeny, biogeography and diversification of the Riodinidae (Lepidoptera: Papilionoidea). Mol Phylogenet Evol 93:296–306. https://doi.org/10.1016/j.ympev.2015.08.006

Farrell BD, Dussourd DE, Mitter C (1991) Escalation of plant defense: do latex and resin canals spur plant diversification? Am Nat 138:881–900

Fine PVA, Metz MR, Lokvam J, Mesones I, Zuñiga J, Lamarre G, Pilco MV, Baraloto C (2013a) Insect herbivores, chemical innovation, and the evolution of habitat specialization in Amazonian trees. Ecology 94:1764–1775. https://doi.org/10.1890/12-1920.1

Fine PVA, Zapata F, Daly DC, Mesones I, Misiewicz TM, Cooper HF, Barbosa C (2013b) The importance of environmental heterogeneity and spatial distance in generating phylogeographic structure in edaphic specialist and generalist tree species of *Protium* (Burseraceae) across the Amazon Basin. J Biogeogr 40:646–661. https://doi.org/10.1111/j.1365-2699.2011.02645.x

Forister ML, Novotny V, Panorska AK, Baje L, Basset Y, Butterill PT, Cizek L, Coley PD, Dem F, Diniz IR, Drozd P, Fox M, Glassmire AE, Hazen R, Hrcek J, Jahner JP, Kaman O, Kozubowski TJ, Kursar TA, Lewis OT, Lill J, Marquis RJ, Miller SE, Morais HC, Murakami M, Nickel H, Pardikes NA, Ricklefs RE, Singer MS, Smilanich AM, Stireman JO, Villamarín-Cortez S, Vodka S, Volf M, Wagner DL, Walla T, Weiblen GW, Dyer LA (2015) The global distribution of diet breadth in insect herbivores. Proc Natl Acad Sci USA 112:442–447. https://doi.org/10.1073/pnas.1423042112

Futuyma DJ, Agrawal AA (2009) Macroevolution and the biological diversity of plants and herbivores. Proc Natl Acad Sci USA 106:18054–18061. https://doi.org/10.1073/pnas.0904106106

Gould F (1991) The evolutionary potential of crop pests. Am Sci 79:496–507

Green PT, Harms KE, Connell JH (2014) Nonrandom, diversifying processes are disproportionately strong in the smallest size classes of a tropical forest. Proc Natl Acad Sci USA 111:18649–18654. https://doi.org/10.1073/pnas.1321892112

Haak DC, Ballenger BA, Moyle LC (2014) No evidence for phylogenetic constraint on natural defense evolution among wild tomatoes. Ecology 95:1633–1641. https://doi.org/10.1890/13-1145.1

Hairston NG, Smith FE, Slobodkin LB (1960) Community structure, population control, and competition. Am Nat 94:421–425

Harms KE, Wright SJ, Calderón O, Hernandez A, Herre EA (2000) Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. Nature 404:493–495. https://doi.org/10.1038/35006630

Janz N (2011) Ehrlich and Raven revisited: mechanisms underlying codiversification of plants and enemies. Annu Rev Ecol Evol Syst 42:71–89. https://doi.org/10.1146/annurev-ecolsys-102710-145024

Janzen DH (1970) Herbivores and the number of tree species in tropical forests. Am Nat 104:501–528

Janzen D (1988) Ecological characterization of a Costa Rican dry forest caterpillar fauna. Biotropica 20:120–135

Jermy T, Szentesi Á (2003) Evolutionary aspects of host plant specialisation—a study on bruchids (Coleoptera: Bruchidae). Oikos 101:196–204. https://doi.org/10.1034/j.1600-0706.2003.11918.x

Koptur S (1984) Experimental evidence for defense of *Inga* (Mimosoideae) saplings by ants. Ecology 65:1787–1793. https://doi.org/10.2307/1937775

Kraft NJB, Godoy O, Levine JM (2015a) Plant functional traits and the multidimensional nature of species coexistence. Proc Natl Acad Sci USA 112:797–802. https://doi.org/10.1073/pnas.1413650112

Kraft NJB, Adler PB, Godoy O, James EC, Fuller S, Levine JM (2015b) Community assembly, coexistence and the environmental filtering metaphor. Funct Ecol 29:592–599. https://doi.org/10.1111/1365-2435.12345

Kursar TA, Coley PD (1991) Nitrogen content and expansion rate of young leaves of rain forest species: implications for herbivory. Biotropica 23:141–150. https://doi.org/10.2307/2388299

Kursar T, Coley PD (1992a) The consequences of delayed greening during leaf development for light absorption and light use efficiency. Plant Cell Environ 15:901–909. https://doi.org/10.1111/j.1365-3040.1992.tb01022.x

Kursar TA, Coley PD (1992b) Delayed development of the photosynthetic apparatus in tropical rain forest species. Funct Ecol 6:411–422. https://doi.org/10.2307/2389279

Kursar TA, Coley PD (1992c) Delayed greening in tropical leaves: an antiherbivore defense? Biotropica 24:256–262. https://doi.org/10.2307/2388520

Kursar TA, Coley PD (2003) Convergence in defense syndromes of young leaves in tropical rainforests. Biochem Syst Ecol 31:929–949. https://doi.org/10.1016/S0305-1978(03)00087-5

Kursar TA, Wolfe BT, Epps MJ, Coley PD (2006) Food quality, competition, and parasitism influence feeding preference in a neotropical lepidopteran. Ecology 87:3058–3069. https://doi.org/10.1890/0012-9658(2006)87[3058:FQCAPI]2.0.CO;2

Kursar TA, Dexter KG, Lokvam J, Pennington RT, Richardson JE, Weber MG, Murakami ET, Drake C, McGregor R, Coley PD (2009) The evolution of antiherbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. Proc Natl Acad Sci USA 106:18073–18078. https://doi.org/10.1073/pnas.0904786106

LaManna JA, Mangan SA, Alonso A, Bourg NA, Brockelman WY, Bunyavejchewin S, Chang L-W, Chiang J-M, Chuyong GB, Clay K, Condit R, Cordell S, Davies SJ, Furniss TJ, Giardina CP, Gunatilleke IAUN, Gunatilleke CVS, Fangliang H, Howe RW,

Hubbell SP, Hsieh C-F, Inman-Narahari FM, Janík D, Johnson DJ, Kenfack D, Korte L, Král K, Larson AJ, Lutz JA, McMahon SM, McShea WJ, Memiaghe HR, Nathalang A, Novotny V, Ong PS, Orwig DA, Ostertag R, Parker GC, Phillips RP, Sack L, Sun I-F, Tello JS, Thomas DW, Turner BL, Vela Díaz DM, Vrška T, Weiblen GD, Wolf A, Yap S, Myers JA (2017) Plant diversity increases with the strength of negative density dependence at the global scale. Science 356:1389–1392. https://doi.org/10.1126/science.aam5678

Lamarre GPA, Mendoza I, Fine PVA, Baraloto C (2014) Leaf synchrony and insect herbivory among tropical tree habitat specialists. Plant Ecol 215:209–220. https://doi.org/10.1007/s11258-013-0290-y

Lavin M (2006) Floristic and geographic stability of discontinuous seasonally dry tropical forests explains patterns of plant phylogeny and endemism. In: Pennington RT, Ratter JA, Lewis GP (eds) Neotropical savannas and seasonally dry forests: plant biodiversity, biogeographic patterns and conservation. CRC Press, Boca Raton, pp 433–447

Léotard G, Saltmarsh A, Kjellberg F, McKey D (2008) Mutualism, hybrid inviability and speciation in a tropical ant–plant. J Evol Biol 21:1133–1143. https://doi.org/10.1111/j.1420-9101.2008.01521.x

Lill JT, Marquis RJ (2001) The effects of leaf quality on herbivore performance and attack from natural enemies. Oecologia 126:418–428. https://doi.org/10.1007/s004420000557

Lokvam J, Kursar TA (2005) Divergence in structure and activity of phenolic defenses in young leaves of two co-occurring *Inga* species. J Chem Ecol 31:2563–2580. https://doi.org/10.1007/s10886-005-7614-x

Lokvam J, Coley PD, Kursar TA (2004) Cinnamoyl glucosides of catechin and dimeric procyanidins from young leaves of *Inga umbellifera* (Fabaceae). Phytochemistry 65:351–358. https://doi.org/10.1016/j.phytochem.2003.11.012

Lokvam J, Brenes-Arguedas T, Lee JS, Coley PD, Kursar TA (2006) Allelochemic function for a primary metabolite: the case of L-tyrosine hyper-production in *Inga umbellifera* (Fabaceae). Am J Bot 93:1109–1115. https://doi.org/10.3732/ajb.93.8.1109

Lokvam J, Metz MR, Takeoka GR, Nguyen L, Fine PV (2015) Habitat-specific divergence of procyanidins in *Protium subserratum* (Burseraceae). Chemoecology 25:293–302. https://doi.org/10.1007/s00049-015-0198-1

Marquis RJ, Salazar D, Baer C, Reinhardt J, Priest G, Barnett K (2016) Ode to Ehrlich and Raven or how herbivorous insects might drive plant speciation. Ecology 97:2939–2951. https://doi.org/10.1002/ecy.1534

Mertens J, Van Moerkercke A, Vanden Bossche R, Pollier J, Goossens A (2016) Clade IVa basic helix–loop–helix transcription factors form part of a conserved jasmonate signaling circuit for the regulation of bioactive plant terpenoid biosynthesis. Plant Cell Physiol 57:2564–2575

Mitter C, Brooks DR (1983) Phylogenetic aspects of coevolution. In: Futuyma DM, Slatkin M (eds) Coevolution. Sinauer Associates, Sunderland, pp 65–98

Nosil P (2012) Ecological speciation. Oxford University Press, Oxford

Novotny V, Basset Y, Miller S, Kitching R, Laidlaw M, Drozd P, Cizek L (2004) Local species richness of leaf-chewing insects feeding on woody plants from one hectare of a lowland rainforest. Conserv Biol 18:227–237

Pennington TD (1997) The genus *Inga*. Royal Botanic Gardens, Kew

Poorter L (2007) Are species adapted to their regeneration niche, adult niche, or both? Am Nat 169:433–442. https://doi.org/10.1086/512045

Richards LA, Coley PD (2007) Seasonal and habitat differences affect the impact of food and predation on herbivores: a comparison

between gaps and understory of a tropical forest. Oikos 116:31–40. https://doi.org/10.1111/j.2006.0030-1299.15043.x

Richards LA, Dyer LA, Forister ML, Smilanich AM, Dodson CD, Leonard MD, Jeffrey CS (2015) Phytochemical diversity drives plant–insect community diversity. Proc Natl Acad Sci USA 112:10973–10978. https://doi.org/10.1073/pnas.1504977112

Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM (2001) Rapid diversification of a species-rich genus of neotropical rain forest trees. Science 293:2242–2245. https://doi.org/10.1126/science.1061421

Roslin T, Hardwick B, Novotny V, Petry WK, Andrew NR, Asmus A, Barrio IC, Basset Y, Boesing AL, Bonebrake TC, Cameron EK, Dattilo W, Donoso DA, Drozd P, Gray CL, Hik DS, Hill SJ, Hopkins T, Huang S, Koane B, Laird-Hopkins B, Laukkanen L, Lewis OT, Milne S, Mwesige I, Nakamura A, Nell CS, Nichols E, Prokurat A, Sam K, Schmidt NM, Slade A, Slade V, Suchankova A, Teder T, van Nouhuys S, Vandvik V, Weissflog A, Shukovich V, Slade E (2017) Higher predation risk for insect prey at low latitudes and elevations. Science 356:742–744. https://doi.org/10.1126/science.aaj1631

Ryan S, Cane K, DeBoer K, Sinclair S, Brimblecombe R, Hamill J (2012) Structure and expression of the quinolinate phosphoribosyltransferase (QPT) gene family in *Nicotiana*. Plant Sci 188:102–110. https://doi.org/10.1016/j.plantsci.2012.02.008

Salazar D, Jaramillo MA, Marquis RJ (2016a) Chemical similarity and local community assembly in the species rich tropical genus *Piper*. Ecology 97:3176–3183. https://doi.org/10.1002/ecy.1536

Salazar D, Jaramillo MA, Marquis RJ (2016b) The impact of plant chemical diversity on plant–herbivore interactions at the community level. Oecologia 181:1199–1208. https://doi.org/10.1007/s00442-016-3629-y

Schemske DW (2009) Biotic interactions and speciation in the tropics. In: Butlin RK, Bridle JR, Schluter D (eds) Speciation and patterns of diversity. Cambridge University Press, Cambridge, pp 219–239

Schluter D (2000) The ecology of adaptive radiation. Oxford University Press, Oxford

Sedio BE (2013) Trait evolution and species coexistence in the hyperdiverse tropical forest tree genus *Psychotria*, Ph.D. dissertation, Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan, USA

Sedio BE (2017) Recent breakthroughs in metabolomics promise to reveal the cryptic chemical traits that mediate plant community composition, character evolution and lineage diversification. New Phytol 214:952–958. https://doi.org/10.1111/nph.14438

Sedio BE, Wright SJ, Dick CW (2012) Trait evolution and the coexistence of a species swarm in the tropical forest understorey. J Ecol 100:1183–1193. https://doi.org/10.1111/j.1365-2745.2012.01993.x

Sedio BE, Echeverri JCR, Boya P, Cristopher A, Wright SJ (2017) Sources of variation in foliar secondary chemistry in a tropical forest tree community. Ecology 98:616–623. https://doi.org/10.1002/ecy.1689

Segasothy M, Swaminathan M, Kong NCT, Bennett WM (1995) Djenkol bean poisoning (djenkolism): an unusual cause of acute renal failure. Am J Kidney Dis 25:63–66. https://doi.org/10.1016/0272-6386(95)90627-4

Singer MS, Stireman JO (2005) The tri-trophic niche concept and adaptive radiation of phytophagous insects. Ecol Lett 8:1247–1255. https://doi.org/10.1111/j.1461-0248.2005.00835.x

Sinimbu G, Coley PD, Lemes MR, Lokvam J, Kursar TA (2012) Do the antiherbivore traits of expanding leaves in the neotropical tree *Inga paraensis* (Fabaceae) vary with light availability? Oecologia 170:669–676. https://doi.org/10.1007/s00442-012-2353-5

Terborgh J (2012) Enemies maintain hyperdiverse tropical forests. Am Nat 179:303–314. https://doi.org/10.1086/664183

Thompson JN (1988) Coevolution and alternative hypothesis on insect/plant interactions. Ecology 69:893–895

Valencia R, Condit R, Foster RB, Romoleroux K, Munoz GV, Svenning J-C, Magard E, Bass M, Losos Balslev H (2004) Yasuní forest dynamics plot, Ecuador. In: Losos E, Leigh J, Giles E (eds) Tropical forest diversity and dynamism: findings from a large-scale plot network. University of Chicago Press, Chicago, pp 609–620

van Schaik CP, Terborgh JW, Wright SJ (1993) The phenology of tropical forests: adaptive significance and consequences for primary consumers. Ann Rev Ecol Syst 24:353–377. https://doi.org/10.1146/annurev.es.24.110193.002033

Wahlberg N (2001) The phylogenetics and biochemistry of host-plant specialization in Melitaeine butterflies (Lepidoptera: Nymphalidae). Evolution 55:522–537. https://doi.org/10.1111/j.0014-3820.2001.tb00786.x

Wahlberg N, Wheat CW, Peña C (2013) Timing and patterns in the taxonomic diversification of Lepidoptera (butterflies and moths). PLoS One 8:e80875

Wiggins NL, Forrister DL, Endara MJ, Coley PD, Kursar TA (2016) Quantitative and qualitative shifts in defensive metabolites define chemical defense investment during leaf development in *Inga*, a genus of tropical trees. Ecol Evol 6:478–492. https://doi.org/10.1002/ece3.1896

Wolda H (1988) Insect seasonality: Why? Annu Rev Ecol Syst 19:1–18. https://doi.org/10.1146/annurev.es.19.110188.000245

Wray GA (2007) The evolutionary significance of *cis*-regulatory mutations. Nat Rev Gen 8:206–216. https://doi.org/10.1038/nrg2063

Wright JS (2002) Plant diversity in tropical forests: a review of mechanisms of species coexistence. Oecologia 130:1–14. https://doi.org/10.1007/s004420100809

Zhu K, Woodall CW, Monteiro JVD, Clark JS (2015) Prevalence and strength of density-dependent tree recruitment. Ecology 96:2319–2327. https://doi.org/10.1890/14-1780.1