Coevolutionary arms race versus host defense chase in a tropical herbivore–plant system

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Coevolutionary models suggest that herbivores drive diversification and community composition in plants. For herbivores, many questions remain regarding how plant defenses shape host choice and community structure. We addressed these questions using the tree genus *Inga* and its lepidopteran herbivores in the Amazon. We constructed phylogenies for both plants and insects and quantified host associations and plant defenses. We found that similarity in herbivore assemblages between *Inga* species was correlated with similarity in defenses. There was no correlation with phylogeny, a result consistent with our observations that the expression of defenses in *Inga* is independent of phylogeny. Furthermore, host defensive traits explained 40% of herbivore community similarity. Analyses at finer taxonomic scales showed that different lepidopteran clades select hosts based on different defenses, suggesting taxon-specific histories of herbivore–host plant interactions. Finally, we compared the phylogeny and defenses of *Inga* to phylogenies for the major lepidopteran clades. We found that closely related herbivores fed on *Inga* with similar defenses rather than on closely related plants. Together, these results suggest that plant defenses might be more evolutionarily labile than the herbivore traits related to host association. Hence, there is an apparent asymmetry in the evolutionary interactions between *Inga* and its herbivores. Although plants may evolve under selection by herbivores, we hypothesize that herbivores may not show coevolutionary adaptations, but instead "chase" hosts based on the herbivore's own traits at the time that they encounter a new host, a pattern more consistent with resource tracking than with the arms race model of coevolution.

coevolution | defensive traits | herbivores | *Inga* | plant–herbivore interactions

Because plants and their insect enemies are strikingly species-rich groups, understanding their interactions is a foundational issue in ecology and evolution. Coevolutionary theory has long predicted that the arms race between plants and herbivores is the principal explanation for this great diversity (1). Coevolutionary and escape-and-radiate models suggest that herbivores might drive speciation in plants (1, 2). A number of recent, independent studies suggest that herbivore pressure contributes to the high local plant diversity, or coexistence, that is typical of plant communities in tropical rainforests (3–6). For herbivores, however, many questions remain with respect to factors shaping community structure, diversification, and coevolution. To begin to address these questions, we must understand the extent to which host choice is evolutionarily conserved. Although plant antiherbivore traits play a prominent role in determining host choice and need not track plant phylogeny, antiherbivore defenses are often not sufficiently considered. Here, we test hypotheses about herbivore host selection by extensively characterizing defenses of a speciose genus of trees co-occurring at one site, and by comparing phylogenies for both trophic groups.

The seminal work of Ehrlich and Raven (1) suggested that plants and insects reciprocally produce evolutionary change. This model predicts that evolutionary constraints (hereafter phylogenetic conservatism) will lead to phylogenetic signal for traits related to their interactions, for both hosts and herbivores. In other words, closely related plant species would have similar defenses and closely related herbivores would feed on closely related plants. Thus, the relationship between plants and herbivores, at both ecological and evolutionary levels, is expected to be strongly phylogenetically structured.

The Ehrlich and Raven model, and many subsequent studies, consider macroevolutionary processes across genera and families (7, 8). At these levels, phylogeny may be a good proxy for shared traits, and many resource acquisition traits show a phylogenetic signal. However, recent work at the species level suggests that herbivores have selected for divergence in defenses in closely related host species. Specifically, studies within several plant genera have found a poor pattern of congruence between their phylogenetic histories and the expression of defenses (3, 5, 6, 9, 10). Furthermore, within a community, neighboring plants are more likely to differ in defenses than expected by chance even if they are closely related (3, 5, 6). Following the notion that

Significance

Although plants and their herbivores account for most of macroscopic, terrestrial biodiversity, we do not fully understand the evolutionary origins of this high diversity. Coevolutionary theory proposes that adaptations between plants and their herbivores are reciprocal and that their interactions might have driven diversification and community composition. Contrary to this scenario of defense and counterdefense, we find an apparent asymmetry in the interactions between plants and herbivores. Specifically, despite the evolutionary constraints of long lifetimes for trees, plant–antiherbivore defenses may be more evolutionarily labile than herbivore adaptations to their hosts, allowing long-lived plant species to persist in the arms race with their insect herbivores. In contrast, herbivores may be evolutionarily "chasing" plants, feeding on species for which they have preadaptations.

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herbivores track or "chase" host defenses and not host species per se (11, 12), we would expect host choice at the level of plant species to mirror host defenses more than host phylogeny, a pattern that would diminish the role of plant phylogenetic relationships in the origin and structure of herbivore communities.

These predictions suggest that, in addition to phylogeny, focusing on ecologically relevant traits for host selection, such as plant defenses, is central to critical evaluation of the various hypotheses relating plant–insect interactions to community composition and diversity. These hypotheses must incorporate the multiple defenses used by plants (mechanical, developmental, phenological, biotic, chemical), and the diverse assemblages of herbivores that exert disparate selective pressures on their hosts. Moreover, we must consider that different defenses can evolve independently. This would provide a high-dimensional niche space for plants and herbivores, with substantial potential for adaptive radiation in both trophic groups. Therefore, understanding traits and their evolution at the level of species can deliver important insights into the processes structuring plant and herbivore communities.

Here, we test the role of plant–insect interactions in shaping herbivore host association and community structure. We focus our study on the species-rich neotropical tree genus *Inga* (Fabaceae, subfamily Mimosoideae) and its associated herbivores at Los Amigos Research Center, located in the lowland Amazon region of Madre de Dios, Peru. *Inga* includes ~300 described species and occurs in moist and wet forests throughout the New World tropics. In Los Amigos and many neotropical forests, *Inga* constitutes one of the most diverse and abundant tree genera. For example, in 25 ha of forest in Amazonian Ecuador, there are >40 *Inga* species representing 6% of stems >1 cm (13).

We previously reported that defensive traits in *Inga* diverge among close relatives as well as among neighbors (3). Although these results suggest that herbivores may affect evolutionary change and local community assembly composition in *Inga*, much remains unknown regarding *Inga*'s natural enemies. To address this gap, we investigated the interactions between *Inga* and its herbivores by asking the following: (*i*) Are different antiherbivore traits of leaves evolving independently? (*ii*) Do *Inga* defensive traits and/or phylogenetic relationships predict host use by herbivores? (*iii*) Do the major lepidopteran clades that feed on *Inga* differ in their relationships to *Inga* traits and *Inga* phylogeny? (*iv*) Are closely related herbivores feeding on closely related plants?

At Los Amigos, we characterized the defensive traits of expanding leaves for 33 species of *Inga*. We focused on expanding leaves as the majority of leaf damage occurs during this short window before leaves toughen (14). We included multiple classes of antiherbivore traits to capture as complete an understanding of the entire defensive profile as possible. We recorded the presence of defensive compounds, particularly several different classes of flavonoids, tannins, saponins, and metabolites containing amines. Total production of secondary metabolites in *Inga* comprises about 40–50% of leaf dry weight (15). Detrimental effects for lepidopteran herbivores have been observed in the laboratory at 0.5–2% of diet for whole-leaf extracts and specific fractions, suggesting that these metabolites are highly toxic (16–19). We also characterized the broader defense phenotype of each *Inga* species in terms of physical defenses (length and density of the nonglandular trichomes), biotic defenses (the number and identity of protective ants visiting the leaves), developmental defenses (leaf expansion rate and chloroplast development; ref. 20), and phenological defenses (timing and synchrony in leaf production; Fig. S1; refs. 21 and 22). Leaf expansion rate and chloroplast development (developmental defenses) have been recognized as adaptations that minimize vulnerability to herbivores (20, 22–24). More rapid expansion shortens the vulnerable period when leaves are tender and preferred by herbivores (22). Species with delayed chloroplast development have lower concentrations of energy and nitrogen and

thus lose fewer resources per given amount of herbivory (22–24). Synchronization and timing of leaf production (phenological defenses) have been shown to be important defensive strategies (21, 22, 25). Species may synchronize leaf production at a population level within species to satiate herbivores (21). Meanwhile, temporal separation of leaf production among species may be favored as a strategy for partial escape from herbivory (20).

We DNA barcoded and quantified the abundance of Lepidoptera associated with the expanding leaves of each *Inga* species, and developed multilocus phylogenies for the most abundant lepidopteran clades, the superfamily Gelechioidea, and the families Erebidae and Riodinidae. We also developed a multilocus phylogeny for *Inga*. We use these data and phylogenetic hypotheses to address how, for plant species within a single genus and at a single site, antiherbivore traits influence the assembly of the herbivore community.

Results and Discussion

Are Different Antiherbivore Traits of Leaves Evolving Independently?
Most of the antiherbivore traits we measured show weak and nonsignificant correlations across species, with a few key exceptions (Table S1). Species of *Inga* with a higher density of leaf trichomes also exhibit longer trichomes (physical defenses, $r^2 = 0.74$, $P < 0.001$). Rapid leaf expansion correlates with lower chlorophyll content (developmental defenses, $r^2 = -0.53$, $P < 0.01$) as has been found in other studies (3, 22). Species that were more similar in the mean number of ants visiting the extrafloral nectaries, were also visited by similar species of ants (biotic defenses, partial Mantel test controlling for phylogenetic relatedness, $r = 0.28$, $P = 0.02$).

We performed a phylogenetic principal-component analysis (PPCA) on traits represented by continuous data to test the hypothesis that different defense categories evolve independently (i.e., are orthogonal in trait space). Consistent with the trait correlation analyses, PPCA determined five significant axes of defense variation [eigenvalues >0.7; Jolliffe cutoff (26)], with each axis being highly correlated with a different defense mechanism. The first axis was highly correlated with trichome density and length (physical defenses, $r = 0.94$ for both traits), the second axis with leaf expansion rate and chlorophyll content (developmental defenses, $r = 0.67$ and $r = -0.81$, respectively), the third axis with timing of leaf production (phenological defense, $r = 0.87$), the fourth axis with the mean number of ants visiting extrafloral nectaries (biotic defenses, $r = 0.73$), and the last axis with synchrony in leaf production ($r = -0.68$).

Because none of the PPCA-derived axes was correlated with chemical defenses (Table S2), antiherbivore traits in *Inga* clearly fall into six independent axes of defense expression or categories: physical, developmental, biotic, timing, synchrony, and chemical. Given that each defense category varies largely independently of the others, plants may have many axes of trait divergence. Despite the possibility that some trait combinations may be missing due to trade-offs or physiological constraints, it seems very likely that the defensive phenotypes of plants can respond to selection in complex ways. This would support the hypothesis that antiherbivore defenses may provide a highly dimensional niche space in which many species of plants and herbivores, some of which are otherwise ecologically similar, are distinctive and can stably co-occur.

Do *Inga* Defensive Traits and/or Phylogenetic Relationships Predict Host Use by Herbivores?
Although *Inga* species host a diversity of herbivores, we focus on the Lepidoptera because extensive field observations demonstrate that these are responsible for most of the damage to expanding leaves. The herbivore community was characterized with a sample of 1,576 individuals comprising 174 molecular operational taxonomic units (MOTUs) based on DNA sequences for the widely used cytochrome oxidase *c*
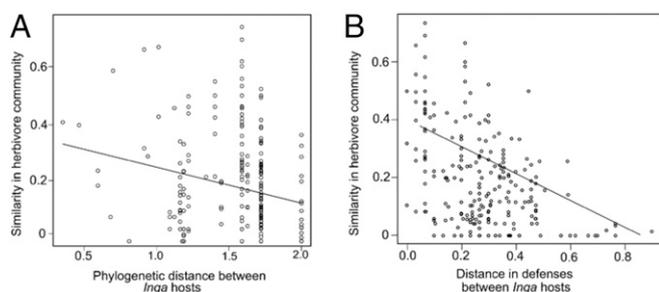
Fig. 1. Relationship between the similarity of lepidopteran communities (1, Bray–Curtis index) on host plants vs. (*A*) phylogenetic distance between *Inga* hosts and (Mantel $r = -0.25$, $P = 0.02$), (*B*) distance in defenses between *Inga* hosts for all pairwise combinations of plants (partial Mantel $r = -0.50$, $P = 0.001$).

subunit 1 barcode. These comprise representatives of 19 families of Lepidoptera, feeding on 33 *Inga* species (Fig. S2).

We determined whether differences in total herbivore assemblages for pairs of *Inga* hosts were related to *Inga* phylogeny and/or defensive traits. Matrix correlation analyses using the entire sample of Lepidoptera reveals a negative relationship of assemblage similarity with host phylogenetic distance, such that more closely related *Inga* species showed greater lepidopteran community similarity (partial Mantel $r = -0.25$, $P = 0.02$; Fig. 1*A* and Table 1). We also found a negative correlation between assemblage similarity and defense distance between *Inga* hosts, such that *Inga* species with similar defenses are attacked by similar herbivores (partial Mantel $r = -0.50$, $P = 0.01$; Fig. 1*B* and Table 1). The much higher correlation in the partial Mantel test for defenses indicates that similarity in defensive traits between *Inga* species predicts host associations for lepidopteran herbivores much more strongly than phylogenetic relatedness of *Inga*.

To quantify the extent to which host phylogeny and/or host defenses structure associated herbivore assemblages, and determine which host traits are the strongest predictors (i.e., most important), we also performed distance-based redundancy analyses (dbRDAs). The dbRDAs showed that plant defensive traits explained much of the variation in the lepidopteran assemblage ($R^2_{adj} = 0.40$, $P = 0.001$). Thirty percent of the total variation was explained solely by host chemistry (chemistry $R^2_{adj} = 0.31$, $P = 0.001$), with an additional 6% explained by physical defenses (trichome density and length $R^2_{adj} = 0.06$, $P = 0.02$). Neither host phylogeny nor the other four defenses were selected as significant variables.

The ordination diagram of the herbivore assemblages associated with each *Inga* species (a grouping of *Inga* hosts in "herbivore space"; Fig. 2) supported these findings by clustering assemblages from *Inga* species that share similar secondary metabolite profiles. This result is of particular interest because, in contrast to previous studies including only a few compounds characteristic of particular species, genera, or families, our approach covered a range of chemical defenses, including saponins, flavonoids, tannins, and diverse amines. This suggests that studies with broader analyses of plant metabolites could be highly instructive.

Do the Major Lepidopteran Clades That Feed on *Inga* Differ in Their Relationship to *Inga* Traits and *Inga* Phylogeny? The Gelechioidea, Erebidae, and Riodinidae are the major lepidopteran clades feeding on young *Inga* leaves, comprising 52% of the species and 81% of the individuals found (Fig. S2). First, we examined whether these clades differed in their responses to *Inga* traits vs. *Inga* phylogeny. For all clades, matrix correlation analyses showed that the similarity in herbivore assemblage consistently decreases with increasing defense distance between *Inga* species (Table 1), and significantly so in two of three datasets. In contrast, plant relatedness had a significant effect on host choice

only for the superfamily Gelechioidea, with similarity in herbivore assemblage decreasing with increasing phylogenetic distance between *Inga* species (Table 1). Host use by Riodinidae and Erebidae was not predicted by plant phylogeny (Table 1).

Variation partitioning analyses revealed that different groups of herbivores are associated with different host traits. For example, Gelechioidea are distinct from the other two clades in that, for most species, the larvae minimize predation by concealment, either by leaf-mining or by hiding between leaves bound together with silk. For this group, plant secondary metabolites ($R^2_{adj} = 0.23$, $P = 0.02$) were selected as the best predictor (Fig. 3), with higher abundance on *Inga* species that express saponins. To a lesser degree, phylogenetic relationships between *Inga* hosts ($R^2_{adj} = 0.16$, $P = 0.005$) also predicted host association. Because phylogeny is a synthetic measure for phylogenetically conserved traits, these results suggest that other conserved nutritive or defensive traits, not included in this study, are also important predictors of host association for this group of herbivores. Developmental defense was marginally significant, with Gelechioidea associated with species with a relatively slow rate of leaf expansion. Variation in leaf development could affect larvae survival, particularly for species that require longer periods of time for successful development and are confined to a single leaf during their entire larval stage.

In contrast, for Riodinidae, phylogenetic relationships between *Inga* hosts were not a significant predictor. Instead, riodinids were more abundant on those *Inga* that receive greater ant visitation, with biotic defenses explaining as much as 30% of the total variation in community similarity ($R^2_{adj} = 0.29$, $P = 0.014$; Fig. 3). Given that ants commonly prey on caterpillars, this is unusual. However, the larvae of most Riodinidae minimize predation by recruiting ant bodyguards in exchange for honeydew secreted by the larvae; riodinid larvae are, in fact, myrmecophiles (27). Hence, a strong positive effect of ants on host selection and larval survival in Riodinidae is expected. Leaf chemistry also played a significant role in host associations; as for Gelechioidea, the preferred species were defended by saponins ($R^2_{adj} = 0.27$, $P = 0.04$; Fig. 3). Trichomes were marginally significant, with higher abundance on *Inga* species with more trichomes.

For Erebidae, phylogenetic relationships between *Inga* hosts were again not significant, but leaf chemistry, specifically amine-containing compounds ($R^2_{adj} = 0.14$, $P = 0.01$), and phenology were important predictors of abundance of Erebidae (Fig. 3). The phenology of young leaf production, including both the degree of synchrony and, for synchronous species, the date of their leaf flush, function as defenses (21). These plant traits restrict access of leaves to herbivores and are predicted to influence specialization of young-leaf feeders. Our analysis of Erebidae strongly supports this hypothesis, with a significant effect of peak month of leaf flush ($R^2_{adj} = 0.13$, $P = 0.04$). In particular, Erebidae preferred *Inga* species with flushing peaks in June–July and October–November, the beginning of the dry and wet seasons, respectively, over species that flushed at other times of the year. Moreover, only species of *Inga* that express amines had peaks in leaf production at around the same times of the year (partial Mantel $r = 0.12$, $P = 0.02$; Fig. S3), in episodes that are synchronous within species and staggered

Table 1. Summary statistics for the relationship between herbivore communities and host plant traits

Host plant traits	All herbivores	Gelechioidea	Riodinidae	Erebidae
	R	*r*	*r*	*r*
Phylogeny	−0.25*	−0.24*	−0.2	−0.04
All defenses	−0.50*	−0.42*	−0.33*	−0.16

r represents the Mantel and partial Mantel correlations between the dissimilarity in host plant traits and their herbivore communities measured by the Bray–Curtis index. Significant values ($P < 0.05$) are marked with an asterisk.
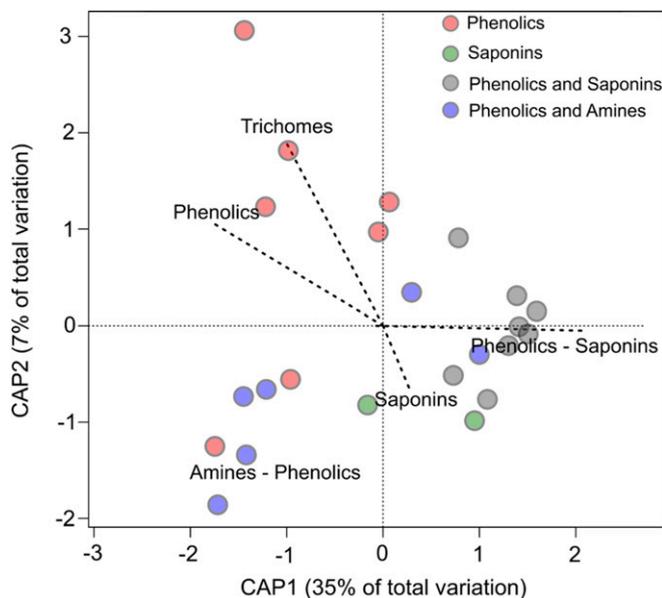
Fig. 2. Distance-based redundancy analysis plot of the most parsimonious model for the lepidopteran community similarity measured by the Bray–Curtis index (R^2_{adj} = 0.40, P = 0.001). Each point represents an *Inga* species and is color-coded by defense chemistry.

among species. These findings suggest that Erebidae closely track leaf production for their preferred hosts, and that flushing leaves simultaneously at a population level, is a strategy to satiate herbivores (21, 28). Our results reinforce long-standing observations that the key stages in the life cycles of herbivorous invertebrates, such as egg deposition, diapause, migration, and possibly mating, may be synchronized with the availability of their principal resource, expanding leaves (28–32) or developing inflorescences (33).

Although chemistry was important for all three clades, two clades preferred hosts that accumulate saponins, whereas Erebidae preferred amines. Clearly, chemistry alone actually is a complex of traits, many of which may evolve independently. Hence, the total number of orthogonal traits likely exceeds six.

We hypothesize that the differences among lepidopteran clades in which *Inga* defensive traits most influence host associations reflect differences in physiology, ecology, and natural history. These differences appear to be at the level of families; more closely related herbivores feed on suites of plants with similar defenses, whereas herbivore families diverge in terms of which defenses matter most for host choice. This result suggests that host choice may evolve slowly relative to plant defenses.

A final important point regards our result that plant defensive traits have a greater predictive power in explaining host associations than does plant phylogeny and that different herbivores respond to different plant defenses. These results highlight the limitations of using plant phylogeny alone to study the processes structuring herbivore communities. This is especially the case when variation in key defensive traits of local plant assemblages is not tightly correlated with their phylogenetic relationships, which seems to be an emerging pattern for plant communities (5, 6, 10, 34, 35). This underscores the importance of characterizing all antiherbivore traits for understanding the ecology and evolution of host range.

Are Closely Related Herbivores Feeding on Closely Related Plants? At an evolutionary level, our results are consistent with the idea that host defensive traits exert strong constraints on herbivore host choice, while herbivore traits that determine host choice may evolve relatively slowly. For example, the three lepidopteran

families choose hosts based on distinctive sets of host traits and five out of six of the defense categories are important in constraining at least one host association. Nevertheless, we also hypothesize that the ensemble of herbivores attacking a given *Inga* species may change fairly readily as herbivores shift onto or add host species for which they have appropriate adaptations (36). Given low phylogenetic patterning of defensive traits in *Inga*, such a model predicts low topological congruence between *Inga* and herbivore phylogenies. For the three major herbivore clades, we found no indication of significant congruence between the *Inga* and herbivore phylogenies (Fig. 4A), a result further supported by the nonmonophyly of the specific lepidopteran groups associated with *Inga*. For Riodinidae and Erebidae, the species found feeding on *Inga* belong to several genera that are not closely related [e.g., *Nymphidium, Sarota, Synargis* for Riodinidae (37) and *Coenipeta, Helia, Melese, Pelochyta*, among others, for Erebidae (38)]. In addition, several of these species also occur on other genera of host plants. For example, the species of *Nymphidium* commonly found on *Inga* also occur on other legumes such as *Zygia* (Mimosoideae, very closely related to *Inga*), and the more distantly related *Senna* and *Cassia* (Caesalpinioideae; ref. 39, Janzen and Hallwachs, Caterpillars of ACG database: janzen.sas.upenn.edu/index.html). The fact that the sampling in the phylogenies of these two families is likely overdispersed across subclades also helps to interpret the effects of undersampling the herbivore phylogeny. A subsample that contains several lineages that are not closely related will err in the direction of phylogenetic divergence (40), because it would tend to inflate the average phylogenetic distance among herbivore species. Thus, trait conservation within the focal herbivore families that we report likely is a robust pattern.

For Gelechioidea, a poorly understood group with few larval feeding records in the tropics, we do not know whether one or more genera are associated with *Inga*. However, given the published phylogeny (41), the morphologies, and the feeding modes of the larvae that we observed (i.e., external feeders and leaf miners), it seems likely that the Gelechioidea that feed on *Inga* are not closely related.

Our analyses of both phylogenies and defensive traits do not support a model of reciprocal evolutionary change (Fig. 4A). Instead, they are consistent with macroevolutionary tracking of *Inga* defenses; that is, herbivore phylogenies are more significantly associated with a dendrogram of *Inga* defenses than expected by chance (Fig. 4B). More closely related herbivores preferred host species with similar defenses rather than closely related *Inga*. Our results are consistent with reports that the evolution of host use in herbivorous insects seems to be relatively more conserved with respect to host defenses rather than to host
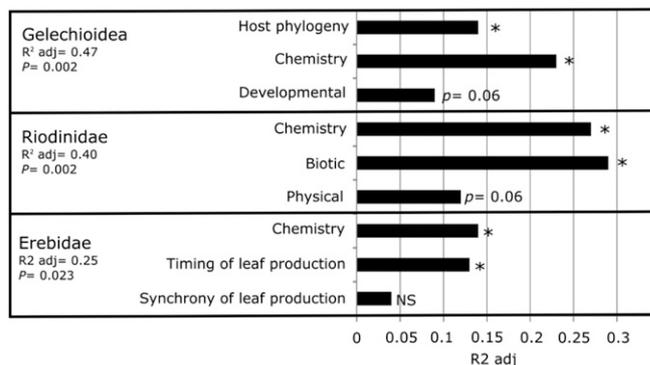

Fig. 3. Results of best-fit distance-based redundancy analyses (dbRDAs) models for the three most abundant lepidopteran families. Significant values ($P < 0.05$) are marked with an asterisk.
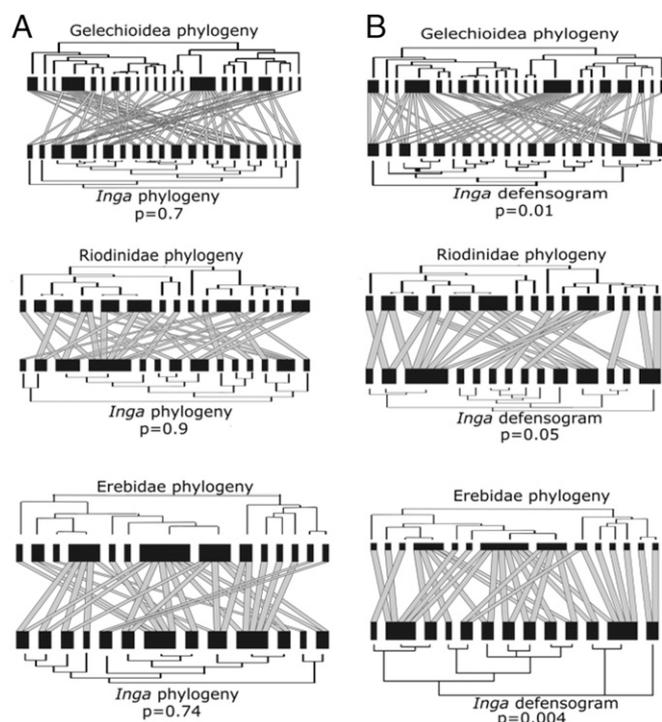
Fig. 4. Bipartite trophic network of *Inga* hosts and herbivores. (*A*) Phylogenies of *Inga* and Lepidoptera plotted in the margins. (*B*) Phylogenies of Lepidoptera and *Inga* defensogram plotted in the margins. For each network, lower bars represent host abundance and upper bars represent herbivore abundance.

phylogeny, not only at the family level (42, 43) but also at finer taxonomic scales (10, 44).

Inga and Its Herbivores: Further Implications.

Do plant–herbivore interactions promote coexistence? The idea that interactions between plants and herbivores may permit high local diversity by favoring coexistence has received considerable theoretical attention and some empirical study. One mechanism could be through increased niche differentiation for both plants and herbivores. Negative, density-dependent interactions with natural enemies could be a principal mechanism structuring plant community assembly because not sharing herbivores with neighbors gives the advantage of reduced damage or "enemy release" (45). Similarly, higher resource partitioning for insect herbivores may narrow niches, especially in tropical forests where herbivores are often highly specialized (46). Previously, we found that *Inga* species that are neighbors in Los Amigos and in Panama differ more in defense strategy than a random draw of the *Inga* community (3), and studies of other species-rich tropical genera, *Bursera*, *Psychotria*, and *Piper*, and at other sites, Mexico and Costa Rica, reveal the same pattern (4–6). Several results from the present study bolster this argument. First, we found that lepidopteran herbivores of *Inga* preferentially forage on subsets of species with similar defensive profiles, even though they are under the same selective pressures and community dynamics, and have the option to select any *Inga* from the community pool as host, suggesting that herbivore associations are constrained by differences in defensive traits. We also found that different groups of herbivores are associated with hosts based on different traits, in ways that make good sense given herbivore biology. Second, we also showed that antiherbivore defenses for *Inga* in Los Amigos fall into at least six independent axes of defense expression, providing a multidimensional niche space for coexistence within which a large number of co-occurring plant and

herbivore species might sort in ecological time (3, 47). Last, as is noted below, plant–herbivore coevolution may be asymmetric, with more labile evolution of plant defenses. As has been suggested for mutualistic networks, the uneven dependency between partners in the interaction may promote stable coexistence (48). Thus, more attention to plant–herbivore interactions has the potential to reveal the mechanisms by which a considerable number of plant species coexist in tropical forests.

Asymmetry in plant vs. herbivore diversification. One long-standing prediction from coevolutionary theory is that defenses of plants and host specificity of insect herbivores should show phylogenetic signal, due to phylogenetic conservatism (1). However, our data do not support this prediction. We find that at least six different classes of defense adaptations can evolve independently. Only trichomes show significant (but not high) phylogenetic signal (Blomberg's $\bar{K} = 0.48$, $P = 0.05$, Table S3) (3), while the other five axes of defense are independent of phylogeny and can be highly divergent among closely related *Inga* species (Fig. S4) (3). Furthermore, herbivore assemblages found on *Inga* species correlate better with host defenses than with host phylogeny (Fig. 1), and host associations for the three most abundant groups of herbivores show phylogenetic relationship with host defenses, but not host phylogeny (Fig. 4). These results strongly suggest that escape from herbivores, associated with rapid evolution of plant defenses (3), has been an important process in the diversification of *Inga*.

In contrast, although adaptations in plant defenses should reduce herbivore fitness, leading to herbivore counteradaptations, the response of the herbivores to selection is less clear. The fact that closely related herbivores attack hosts with similar defense phenotypes rather than closely related ones, imply that herbivores are not tracking species per se but are tracking resources for which they have appropriate adaptations: seasonal activity of females that matches the timing of leaf flushing, host-finding capabilities, avoiding larval predators (particularly ants), a larval period that matches the rate of leaf development, and avoiding the toxic effects of plant chemicals (11). Switches to a novel host with divergent defenses would require that an herbivore rapidly evolve multiple adaptations. However, genetic variation for correlated innovations in a suite of traits is considered improbable (12). If closely related herbivores are similar in the complex set of adaptations to their hosts such that they are constrained to feed on hosts with similar defenses and if plant defenses evolve rapidly, then a pattern of reciprocal diversification seems less likely. Thus, in contrast to a model of a tight coevolutionary process, 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 (refs. 49–52; see ref. 53 for an alternative hypothesis).

This framework suggests that antiherbivore 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. We propose that, despite constraints on rates of adaptation imposed by their long lifetimes, the evolutionary lability of *Inga* defensive traits allows them to persist in the arms race.

Materials and Methods

Study Site. This study was carried out at the Los Amigos Research Center (12°34 S, 70°05 W; elevation, ~270 m) located in a continual expanse of forests between two national parks in the lowland Amazon region of Madre de Dios, Peru. Los Amigos is a conservation concession that comprises 453 ha of primary tropical rainforest on a mixture of upland terraces and floodplains. Annual rainfall at Los Amigos is between 2,700 and 3,000 mm, and the mean monthly temperature ranges from 21 to 26 °C (54).

Characterization of Herbivores and Defensive Traits of *Inga*. Herbivores and defense trait data were collected on expanding leaves from understory saplings of *Inga* species. To record host associations of lepidopteran herbivores,

we visually searched young leaf flushes and collected only those larvae that were found feeding. All larvae, that is, caterpillars, were assigned to morphospecies in the field and subsequently to MOTUs (for MOTU assignment, see *SI Text*) in the laboratory using sequences from the mitochondrial gene cytochrome oxidase I (*COI*). MOTUs were allocated to taxonomic families by searching each consensus sequence against the National Center for Biotechnology Information (NCBI) BLAST web interface, with a minimum accepted similarity for assignment of 90%.

We recorded the presence or absence of several classes of phenolic compounds (10 classes), saponins (1 class), and metabolites containing amines for expanding leaves (3 classes, Table S4). Details on chemical procedures are reported in ref. 3. We assessed the length and density of trichomes per area (number of hairs per 2 cm^2 on the basal leaf surface). Leaf expansion rate was determined as the percent increase in area per day. Chloroplast development was measured as the chlorophyll content (in milligrams per square decimeter) of leaves between 30% and 80% of full expansion. To measure timing and synchrony in leaf production, we monitored between 30 and 70 individuals per tree species for monthly leaf production. To estimate timing in leaf production, we calculated the mean angle (using circular statistics), which indicates the average date of peak flushing activity across all individuals (55). To estimate synchrony in leaf production we calculated the coefficient of variation (CV) of the number of plant individuals per species flushing each month. We also determined the identity and the abundance of ants visiting these nectaries (number of ants per nectary). See *SI Text* for detailed methods.

Phylogenetic Reconstructions. Phylogenetic analyses for MOTUs allocated to the most abundant lepidopteran clades, Gelechioidea, Riodinidae, and Erebidae, were conducted using one to three individuals per MOTU and three gene fragments: nuclear elongation factor (*EF-1α*) and wingless (*Wg*), and mitochondrial *COI*. Phylogenetic relationships were inferred using a multilocus coalescent-based Bayesian species tree approach in *BEAST 2.2.0 (56), with substitution models and codon partition for each marker according to the results of analyses using PartitionFinder 1.1.0 (57). Final phylogenies were derived from three independent runs of 100 million generations combined using LogCombiner 1.8 (58) with a burn-in of 10 million generations and sampling every 10,000 generations in each run. BEAST model convergence was confirmed by examination of parameter estimate distribution in Tracer 1.6. All primer sequences, PCR and sequencing protocols, and details of BEAST model assessment for each clade are in Tables S5–S7.

Phylogenetic relationships among *Inga* host species were inferred using seven chloroplast regions (rpoCI, psbA-trnH, rps16, trnL-F, trnD-T, ndhF-rpl32, rpl32-trnL) and the nuclear ribosomal internal transcribed spacer regions (ITS). PCR and sequencing protocols for chloroplast regions are given by ref. 3 and for ITS by refs. 59 and 60. The phylogeny was estimated using a maximum-likelihood framework using RAxML, with separate models for ITS and cpDNA (61). The phylogeny was subsequently time-calibrated using penalized likelihood (62), where the crown age was constrained to 6 My (following refs. 59–63). Details about DNA extraction and sequencing are in *SI Text*.

From the resulting tree, we extracted pairwise distances between *Inga* species. This phylogenetic distance matrix was used in all of the subsequent ecological analyses that involved the phylogeny of *Inga*. It is important to mention that, although we studied a limited number of species, the *Inga* community in Los Amigos is composed of phylogenetically scattered species (64). Thus, our *Inga* community phylogeny represents a random sampling from the whole-genus phylogeny.

Statistical Analyses.
Relationship between plant traits and phylogenetic signal. Associations between physical, developmental, biotic, and phenological defenses were investigated using phylogenetic generalized least-squares (PGLS) regression (65). To assess

the correlations between chemistry, the community of ants visiting *Inga*, and the other defensive strategies, we used partial Mantel tests, conditioned on a matrix of phylogenetic distances between *Inga* species to control for phylogenetic effects. The distance matrix for biotic (number of ants), developmental, physical, and chemical defenses, as well as synchrony in leaf production were calculated using the Manhattan dissimilarity index. For the ant visitor community, the Bray–Curtis index was used. Chemical dissimilarity between species was based on the presence/absence of secondary compounds (0/1), classified according to their structure. Because the timing in leaf production is a circular variable (mean angle), we used the angular separation method from the package circular (66) to calculate the distance matrix for this trait.

We also performed a phylogenetic PCA on continuous trait data to derive independent axes of defense variation, and to test the hypothesis that different defense phenotypes are able to evolve independently [evolutionary orthogonal in trait space (67)]. Phylogenetic signal was evaluated on the significant axes of defense variation and on the principal coordinates of the chemistry and ant species distance matrices by using Blomberg's $\bar{K}$ (68).
Constraints on host plant selection. Differences in herbivore community structure were related to differences in phylogenetic relationships and/or defensive traits between pairs of *Inga* hosts using partial Mantel tests. Overlap in feeding records among host species was estimated using the Bray–Curtis dissimilarity index with relative abundance data. To quantify the extent to which host phylogeny and/or host defenses structure herbivore community and to determine which defense trait is more important, we used dbRDA with the square-root transformed herbivore community dissimilarity matrix as a response variable together with each one of the measured defensive traits, including chemistry as a dummy variable, and the principal coordinates of the phylogenetic and ant species distance matrix as explanatory variables.
Phylogenetic patterns of host use. To investigate whether host shifts have occurred more often on *Inga* that are more similar in defenses or on *Inga* that are more closely related, we examined the congruence of the herbivore phylogenies with *Inga* phylogeny and *Inga* defenses using ParaFit (69). This statistical tool tests the significance of a hypothesis of congruence between parasites and hosts using distance matrices of associated taxa and a set of host–parasite links. Distances matrices for herbivores and plants were derived from their phylogenetic trees and from a dendrogram (hierarchical clustering) obtained from the total plant defense distance matrix. Model selection for the cluster was based on the correlation between the original distance matrix and the binary matrix representing the partitions in the cluster. The clustering algorithm "UPGMA" showed the highest correlation and hence was selected as the best model for the defenses dendrogram. Significance of the ParaFit test was assessed by permutation. All of the statistical analyses were performed in R Statistical Environment (R Core Developmental Team 2016), and details can be found in *SI Text*.

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