

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

María-José Endara<sup>a,b,1</sup>, Phyllis D. Coley<sup>a,c</sup>, Gabrielle Ghabash<sup>a</sup>, James A. Nicholls<sup>d</sup>, Kyle G. Dexter<sup>e,f</sup>, David A. Donoso<sup>g</sup>, Graham N. Stone<sup>d</sup>, R. Toby Pennington<sup>f</sup>, and Thomas A. Kursar<sup>a,c</sup>

<sup>a</sup>Department of Biology, University of Utah, Salt Lake City, UT 84112-0840; <sup>b</sup>Centro de Investigación de la Biodiversidad y Cambio Climático, Universidad Tecnológica Indoamérica, Quito EC170103, Ecuador; <sup>c</sup>Smithsonian Tropical Research Institute, 0843-03092, Balboa, Ancón, Republic of Panamá; <sup>d</sup>Ashworth Labs, Institute of Evolutionary Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JY, United Kingdom; <sup>e</sup>School of GeoSciences, University of Edinburgh, Edinburgh EH9 3FF, United Kingdom; <sup>f</sup>Royal Botanic Garden Edinburgh, Edinburgh EH3 5LR, United Kingdom; and <sup>g</sup>Instituto de Ciencias Biológicas, Escuela Politécnica Nacional, Quito 17-01-2759, Ecuador

Edited by Douglas Futuyma, Stony Brook University, Stony Brook, NY, and approved July 11, 2017 (received for review May 11, 2017)

**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

**B**ecause 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 life-times 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.**

Author contributions: M.-J.E., P.D.C., and T.A.K. designed research; M.-J.E., P.D.C., G.G., J.A.N., K.G.D., D.A.D., R.T.P., and T.A.K. performed research; M.-J.E., P.D.C., and T.A.K. contributed new reagents/analytic tools; M.-J.E., P.D.C., K.G.D., R.T.P., and T.A.K. analyzed data; and M.-J.E., P.D.C., K.G.D., G.N.S., R.T.P., and T.A.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The novel DNA sequences generated for this paper have been deposited in International Barcode of Life (iBOL) sample IDs: IngaHerbiv0281–IngaHerbiv0908 and RCMJE LA01–RCMJE LA285) and GenBank (accessions nos. [MFS77083–MFS78220](https://doi.org/10.26434/chemrxiv-2017-07-11)). Average values of nonchemical leaf defensive traits are available in [Dataset S1](#), and details about the sequences used for the phylogenies and their associated plant hosts are available in [Dataset S2](#).

<sup>1</sup>To whom correspondence should be addressed. Email: [majo.endara@utah.edu](mailto:majo.endara@utah.edu).

This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1707727114/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1707727114/-DCSupplemental).

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 Erebididae 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?

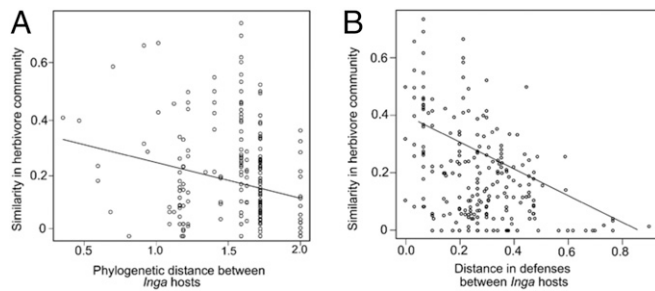
Most of the antiherbivore traits we measured show weak and nonsignificant correlations across species, with a few key exceptions (Table S1). Species of *Inga* with a higher density of leaf trichomes also exhibit longer trichomes (physical defenses,  $r^2 = 0.74$ ,  $P < 0.001$ ). Rapid leaf expansion 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. 1A 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. 1B 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 ( $R^2_{\text{adj}} = 0.40$ ,  $P = 0.001$ ). Thirty percent of the total variation was explained solely by host chemistry (chemistry  $R^2_{\text{adj}} = 0.31$ ,  $P = 0.001$ ), with an additional 6% explained by physical defenses (trichome density and length  $R^2_{\text{adj}} = 0.06$ ,  $P = 0.02$ ). 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 ( $R^2_{\text{adj}} = 0.23$ ,  $P = 0.02$ ) 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 ( $R^2_{\text{adj}} = 0.16$ ,  $P = 0.005$ ) 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 ( $R^2_{\text{adj}} = 0.29$ ,  $P = 0.014$ ; 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 ( $R^2_{\text{adj}} = 0.27$ ,  $P = 0.04$ ; Fig. 3). Trichomes were marginally significant, with higher abundance on *Inga* species with more trichomes.

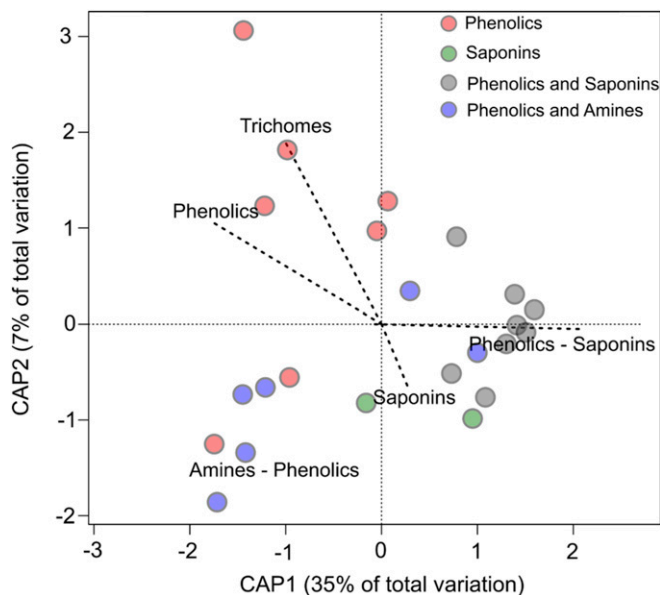
For Erebidae, phylogenetic relationships between *Inga* hosts were again not significant, but leaf chemistry, specifically amine-containing compounds ( $R^2_{\text{adj}} = 0.14$ ,  $P = 0.01$ ), and phenology were important predictors of abundance of Erebidae (Fig. 3). The phenology of young leaf production, 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 ( $R^2_{\text{adj}} = 0.13$ ,  $P = 0.04$ ). 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
	<i>R</i>	<i>r</i>	<i>r</i>	<i>r</i>
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 ( $R^2_{\text{adj}} = 0.40$ ,  $P = 0.001$ ). 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.

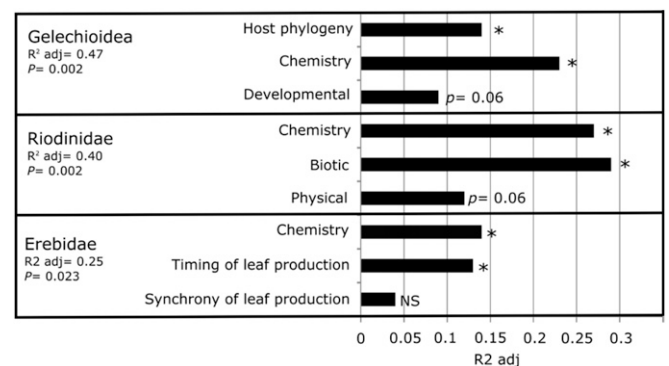
A final important point regards our result that plant defensive traits have a greater predictive power in explaining host associations than does plant phylogeny and that different herbivores respond to different plant defenses. These results highlight the limitations of using plant phylogeny alone to 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

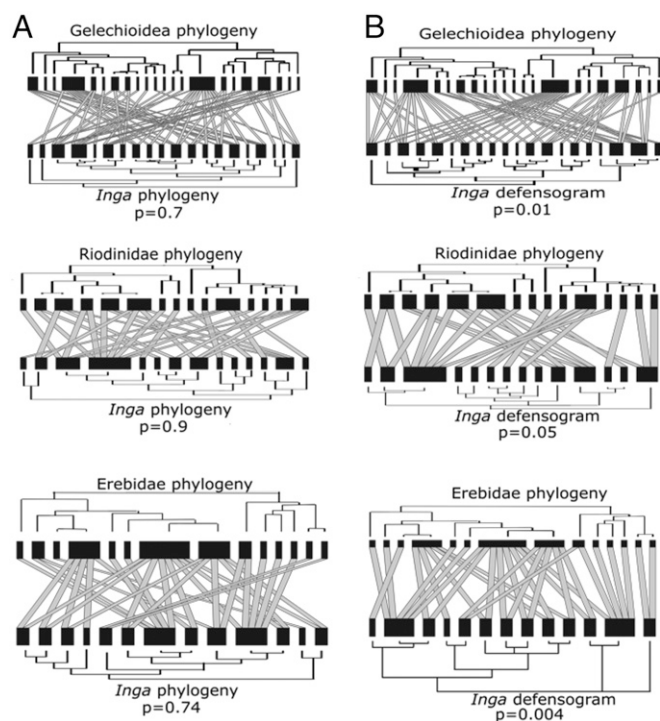
families choose hosts based on distinctive sets of host traits and five out of six of the defense categories are important in constraining at least one host association. Nevertheless, we also hypothesize that the ensemble of herbivores attacking a given *Inga* species may change fairly readily as herbivores 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](http://janzen.sas.upenn.edu/index.html)). The fact that the sampling in the phylogenies of these two families is likely overdispersed across subclasses 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  $K = 0.48$ ,  $P = 0.05$ , 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<sup>2</sup> 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-1 $\alpha$* ) 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 (rpoCl, psbA-trnH, rps16, trnL-F, trnD-T, ndhF-rp132, rp132-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*.

**ACKNOWLEDGMENTS.** We thank the Ministry of Agriculture of Peru for granting the research and exportation permits. We gratefully acknowledge Los Amigos Biological Station for institutional and logistical support. Invaluable field assistance was provided by Wilder Hidalgo and Silvana Lozano. We thank Axel Haussman, Suzy Khachatryan, and Eric Murakami for help in the barcode identification of the insect herbivores. Kyle Harms suggested the short title for the manuscript. This work was supported by the Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación del Ecuador (SENESCYT) and grants from Conservation, Research and Education Opportunities and from the University of Utah: The Global Change and Sustainability Center and the International Student Center from the University of Utah (to M.-J.E.) and National Science Foundation Grants DEB-0640630 and Dimensions of Biodiversity DEB-1135733 (to P.D.C. and T.A.K.).

- Ehrlich PR, Raven PH (1964) Butterflies and plants: A study in coevolution. *Evolution* 18:586–608.
- Marquis RJ, et al. (2016) Ode to Ehrlich and Raven or how herbivorous insects might drive plant speciation. *Ecology* 97:2939–2951.
- Kursar TA, et al. (2009) The evolution of ant herbivore defenses and their contribution to species coexistence in the tropical tree genus *Inga*. *Proc Natl Acad Sci USA* 106:18073–18078.
- Becerra JX (2007) The impact of herbivore-plant coevolution on plant community structure. *Proc Natl Acad Sci USA* 104:7483–7488.
- Sedio B (2013) Trait evolution and species coexistence in the hyperdiverse tropical forest tree genus *Psychotria*, PhD thesis (University of Michigan, Ann Arbor, MI).
- Salazar D, Jaramillo MA, Marquis RJ (2016) Chemical similarity and local community assembly in the species rich tropical genus *Piper*. *Ecology* 97:3176–3183.
- Futuyma DJ, Agrawal AA (2009) Macroevolution and the biological diversity of plants and herbivores. *Proc Natl Acad Sci USA* 106:18054–18061.
- Janz N (2011) Ehrlich and Raven revisited: Mechanisms underlying codiversification of plants and enemies. *Annu Rev Ecol Syst* 42:71–89.
- Agrawal AA, Fishbein M (2006) Plant defense syndromes. *Ecology* 87(7, Suppl): S132–S149.
- Becerra JX (1997) Insects on plants: Macroevolutionary chemical trends in host use. *Science* 276:253–256.
- Brooks DR, McLennan DA (2002) *The Nature of Diversity: An Evolutionary Voyage of Discovery* (Univ of Chicago Press, Chicago).
- Agosta SJ (2006) On ecological fitting, plant-insect associations, herbivore host shifts, and host plant selection. *Oikos* 114:556–565.
- Valencia R, et al. (2004) *Tropical Forest Diversity and Dynamism: Findings from a Large-scale Plot Network*, eds Losos EC, Leigh, Jr EG (Univ of Chicago Press, Chicago), pp 609–628.
- Coley PD, Aide TM (1991) *Plant–Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions*, eds Price PW, Lewinsohn TM, Fernandes WW, Benson WW (Wiley, New York), pp 25–49.



15. Endara MJ, et al. (2015) Divergent evolution in antiherbivore defenses within species complexes at a single Amazonian site. *J Ecol* 103:1107–1118.
16. Coley PD, et al. (2005) Divergent defensive strategies of young leaves in two Neotropical species of *Inga*. *Ecology* 86:2633–2643.
17. 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.
18. Lokvam J, Clausen TP, Grapov D, Coley PD, Kursar TA (2007) Galloyl depsides of tyrosine from young leaves of *Inga laurina*. *J Nat Prod* 70:134–136.
19. Bixenmann RJ, Coley PD, Weinhold A, Kursar TA (2016) High herbivore pressure favors constitutive over induced defense. *Ecol Evol* 6:6037–6049.
20. Kursar TA, Coley PD (1992) The consequences of delayed greening during leaf development for light absorption and light use efficiency. *Plant Cell Environ* 15:901–909.
21. Aide TM (1993) Patterns of leaf development and herbivory in a tropical understorey community. *Ecology* 74:455–466.
22. Kursar TA, Coley PD (2003) Convergence in defense syndromes of young leaves in tropical rainforests. *Biochem Syst Ecol* 21:929–949.
23. Kursar TA, Coley PD (1992) Delayed development of the photosynthetic apparatus in tropical rainforest species. *Funct Ecol* 6:411–422.
24. Kursar TA, Coley PD (1992) Delayed greening in tropical leaves: An anti-herbivore defense? *Biotropica* 24:256–262.
25. Lamarre GPA, Mendoza I, Fine PVA, Baraloto C (2014) Leaf synchrony and insect herbivory among tropical tree habitat specialists. *Plant Ecol* 215:209–220.
26. Jolliffe IT (1986) *Principal Components Analysis* (Springer, New York).
27. Pierce NE, et al. (2002) The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annu Rev Entomol* 47:733–771.
28. Aide TM (1998) Herbivory as a selective agent on the timing of leaf production in a tropical understorey community. *Nature* 336:574–575.
29. Chew FS, Courtney SP (1991) Plant apparency and evolutionary escape from insect herbivory. *Am Nat* 138:729–750.
30. Wolda H (1998) Insect seasonality: Why? *Annu Rev Ecol Syst* 19:1–18.
31. Grøtan V, Lande R, Engen S, Saether BE, DeVries PJ (2012) Seasonal cycles of species diversity and similarity in a tropical butterfly community. *J Anim Ecol* 81:714–723.
32. Srygley RB, Dudley R, Oliveira EG, Riveros AJ (2013) El Niño, host plant growth and migratory butterfly abundance in a changing climate. *Biotropica* 46:90–97.
33. Pratt GF (1994) Evolution of *Euphilotes* (Lepidoptera: Lycaenidae) by seasonal and host shifts. *Biol J Linn Soc Lond* 51:387–416.
34. Pellissier L, et al. (2013) Turnover of plant lineages shapes herbivore phylogenetic beta diversity along ecological gradients. *Ecol Lett* 16:600–608.
35. Whitfield TJS, et al. (2012) Predicting tropical insect herbivore abundance from host plant traits and phylogeny. *Ecology* 93:5211–5222.
36. Menken SBJ (1996) Pattern and process in the evolution of insect-plant associations: *Yponomeuta* as an example. *Entomol Exp Appl* 80:297–305.
37. Espeland M, et al. (2015) Ancient Neotropical origin and recent recolonisation: Phylogeny, biogeography and diversification of the Riodinidae (Lepidoptera: Papilionoidea). *Mol Phylogenet Evol* 93:296–306.
38. Zahirri R, et al. (2012) Molecular phylogenetics of Erebiidae (Lepidoptera, Noctuidae). *Syst Entomol* 37:102–124.
39. DeVries PJ, Chacon IA (1992) Toward a better understanding of host use and biodiversity in riodinid butterflies (Lepidoptera). *J Res Lepid* 31:103–126.
40. Winkler IS, Mitter C (2008) *The Evolutionary Biology of Herbivorous Insects: Specialization, Speciation, and Radiation*, ed Tilmon KJ (Univ of California Press, Berkeley), pp 240–263.
41. Sohn JC, et al. (2015) Phylogeny and feeding trait evolution of the mega-diverse Gelechioidea (Lepidoptera: Obectomera): New insights from 19 nuclear genes. *Syst Entomol* 41:112–132.
42. Berenbaum MR (2001) Chemical mediation of coevolution: Phylogenetic evidence for Apiaceae and associates. *Ann Miss Bot Gard* 88:45–59.
43. Wahlberg N (2001) The phylogenetics and biochemistry of host-plant specialization in Melitaeine butterflies (Lepidoptera: Nymphalidae). *Evolution* 55:522–537.
44. Becerra JX, Venable DL (1999) Macroevolution of insect-plant associations: The relevance of host biogeography to host affiliation. *Proc Natl Acad Sci USA* 96:12626–12631.
45. Yguel B, et al. (2011) Phytophagy on phylogenetically isolated trees: Why hosts should escape their relatives. *Ecol Lett* 14:1117–1124.
46. Dyer LA, et al. (2007) Host specificity of Lepidoptera in tropical and temperate forests. *Nature* 448:696–699.
47. Coley PD, Kursar TA (2014) Ecology. On tropical forests and their pests. *Science* 343:35–36.
48. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science* 312:431–433.
49. Janzen DH (1980) When is coevolution? *Evolution* 34:611–612.
50. Agosta SJ, Klemens JA (2008) Ecological fitting by phenotypically flexible genotypes: Implications for species associations, community assembly and evolution. *Ecol Lett* 11:1123–1134.
51. Berenbaum MR (1990) *Insects Life Cycles: Genetics, Evolution and Co-ordination*, ed Gilbert F (Springer, London), pp 87–99.
52. Bernays E, Graham M (1998) On the evolution of host specificity in phytophagous arthropods. *Ecology* 69:886–892.
53. Jermy T (1984) Evolution of insect/host plant relationships. *Am Nat* 124:609–630.
54. Malhi Y, et al. (2002) An international network to understand the biomass and dynamics of Amazonian forests (RAINFOR). *J Veg Sci* 13:439–450.
55. Zar JH (1999) *Biostatistical Analysis* (Prentice-Hall, Upper Saddle River, NJ).
56. Heled J, Drummond AJ (2010) Bayesian inference of species trees from multilocus data. *Mol Biol Evol* 27:570–580.
57. Lanfear R, Calcott B, Ho SYW, Guindon S (2012) Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol* 29:1695–1701.
58. Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214.
59. 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.
60. Dexter KG, Pennington TD, Cunningham CW (2010) Using DNA to assess errors in tropical tree identifications: How often are ecologists wrong and when does it matter? *Ecol Monogr* 80:267–286.
61. Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690.
62. Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. *Mol Biol Evol* 19:101–109.
63. Lavin M (2006) *Neotropical Savannas and Seasonally Dry Forests: Plant Diversity, Biogeography and Conservation*, eds Pennington RT, Lewis GP, Ratter JA (CRC, Boca Raton, FL), pp 433–447.
64. Dexter KG, et al. (2017) Dispersal assembly of rain forest tree communities across the Amazon basin. *Proc Natl Acad Sci USA* 114:2645–2650.
65. Garland T, Midford PE, Ives AR (1999) An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. *Am Zool* 39:374–388.
66. Lund U, Agostinelli C (2015) Circular: R Package for Circular Statistics. Available at [cran.r-project.org/web/packages/circular/circular.pdf](http://cran.r-project.org/web/packages/circular/circular.pdf). Accessed October 10, 2016.
67. Revell LJ (2009) Size-correction and principal components for interspecific comparative studies. *Evolution* 63:3258–3268.
68. Blomberg SP, Garland T, Jr, Ives AR (2003) Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* 57:717–745.
69. Legendre P, Desdevises Y, Bazin E (2002) A statistical test for host-parasite coevolution. *Syst Biol* 51:217–234.
70. Pennington TD (1997) *The Genus Inga: Botany* (Univ of Chicago Press, Chicago).
71. 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.
72. 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.
73. Brenes-Arguedas T, Coley PD, Kursar TA (2008) Divergence in the chemical ecology of *Inga* between two Neotropical sites. *J Ecol* 96:127–135.
74. 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.
75. Jeffery SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c1, and c2 in higher plants, algae and natural phytoplankton. *Biochem Physiol Pflanz* 167:191–194.
76. West SA, Cook JM, Werren JH, Godfray HCJ (1998) *Wolbachia* in two insect host-parasitoid communities. *Mol Ecol* 7:1457–1465.
77. Ivanova NV, deWaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Mol Ecol Notes* 6:998–1002.
78. deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) *Molecular Biology: Environmental Genetics*, ed Martin C (Humana Press, Totowa, NJ), pp 275–293.
79. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797.
80. Jones M, Ghoorah A, Blaxter M (2011) jMOTU and Taxonomer: Turning DNA Barcode sequences into annotated operational taxonomic units. *PLoS One* 6:e19259.
81. Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol Ecol* 21:1864–1877.
82. Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, LA (IEEE, Piscataway, NJ), pp 1–8.
83. Kass R, Raftery A (1995) Bayes factors. *J Am Stat Assoc* 90:773–795.
84. Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer, version 1.6. Available at [beat.bio.ed.ac.uk/Tracer](http://beat.bio.ed.ac.uk/Tracer). Accessed May 20, 2015.
85. Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12:13–15.
86. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol Biol Evol* 30:772–780.
87. Paradis E, et al. (2015) APE: Analyses of phylogenetics and evolution. Available at [cran.r-project.org/web/packages/ape/ape.pdf](http://cran.r-project.org/web/packages/ape/ape.pdf). Accessed February 15, 2016.
88. Oksanen J, et al. (2015) Vegan: Community ecology package. Available at [cran.r-project.org/web/packages/vegan/vegan.pdf](http://cran.r-project.org/web/packages/vegan/vegan.pdf). Accessed February 15, 2016.
89. Legendre P (2012) *Numerical Ecology* (Elsevier, Oxford).
90. Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astrartes fulgerator*. *Proc Natl Acad Sci USA* 101:14812–14817.
91. Cho S, et al. (1995) A highly conserved nuclear gene for low-level phylogenetics: Elongation factor-1 alpha recovers morphology-based tree for heliothine moths. *Mol Biol Evol* 12:650–656.
92. Brower AVZ, DeSalle R (1998) Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: The utility of wingless as a source of characters for phylogenetic inference. *Insect Mol Biol* 7:73–82.