Supporting Information

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Sample Collection. Ecological data were taken for young leaves on saplings (0.5–2.5 m tall) growing in the understory. More than 50 km of trails were walked regularly to search for plants with young leaves. Panamanian collections were made from March 2001 to November 2004, and Peruvian collections were made from May to December 2007.

Rates of leaf expansion were determined by measuring the area of marked leaves every 1–3 days throughout expansion. Expansion rates were calculated as the percentage increase in area per day for leaves between 20% and 80% of full size. Because of herbivore damage, many leaves had to be excluded so expansion data were based on an average of 13 individual leaves per species. Because temperatures differed by 3.9 °C between the study sites/seasons, we adjusted expansion rates from Peru by a factor of 1.51, equivalent to a Q_{10} of 2.7 that we have measured for respiration in tropical leaves. The number of ants visiting extrafloral nectaries of young leaves was counted (# nectary⁻¹) during censuses along trails between 10 AM and 3 PM for an average of 83 plants per species. Chlorophyll content $(mg·m⁻²)$ was determined for an average of nine young leaves per species estimated to be between 60% and 80% of full size. A known area of leaf tissue was homogenized in 95% ethanol and centrifuged, and absorbances at 663 and 725 nm were measured with a portable spectrophotometer (Milton Roy, Spectronic Mini 20). See [Table S3](http://www.pnas.org/cgi/data/0904786106/DCSupplemental/Supplemental_PDF#nameddest=ST3) for values and sample sizes averaged by species.

Chemical Analyses. Young leaves were collected from understory saplings and were between 10% and 90% of full expansion. Less than 1/3 of the leaves on a single flush were collected to minimize negative impacts to the plant. For each species, leaves were collected from many different plants and stored separately. Most of the leaves collected in Panama were homogenized in 95% ethanol by using a Polytron (Brinkmann Instruments) and then stored at -50 °C until shipped to Utah for analysis. Some samples from Panama and all leaves collected in Peru were dried under vacuum (<1 Torr in Panama or 10 Torr, with silica gel, in Peru) for 36–48 h and then stored at -50 °C in Panama and -15 °C in Peru in doubly sealed plastic bags with silica gel until being shipped to Utah. Extracts of young leaves from 37 *Inga* species from Panama and Peru were analyzed for phenolic and saponin content. Panamanian samples were also analyzed for nonprotein amino acids.

Phylogenetic Reconstruction. PCR and sequencing protocols for trnD-T are given in ref. 1. The psbA-trnH region was amplified and sequenced with primers psbA GTTATGCATGAACGTA-ATGCTC and trnH CGCGCATGGTGGATTCAAATCC. The rps16 regions was amplified and sequenced with primers rps16-F GTGGTAGAAAGCAACGTGCGA and rps16-R TCGG-GATCGAACATCAATTGCAAC. PCR conditions for psbAtrnH and rps16 were: one cycle of 94 °C for 2 min; 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min; one cycle of 72 °C for 10 min. The trnL-F region was amplified and sequenced in two parts by using primer pairs trnL c CGAAATCGGTAGACGCTACG, trnL d GGGGATAGAGG-GACTTGAAC, and trnL e GGTTCAAGTCCCTCTATCCC, trnL f ATTTGAACTGGTGACACGAG. PCR conditions for trnL-F were: one cycle of 94 °C for 4 min; 35 cycles of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 3 min; one cycle of 72 °C for 10 min. The ndhF-rpl32 region was amplified and sequenced

following Shaw et al. (2), and rpoC1 was amplified and sequenced following Hollingsworth et al. (3). For the $\approx 50\%$ of samples that did not produce PCR products for ndhF-rpl32 using Shaw et al. (2) protocols, we developed new primers (forward: GGAGCTGCCATTCCAAAAT; reverse: TTCGCCAATTT-TATCTCTTTTG) and new PCR conditions: one cycle of 94 °C for 2 min; 30 cycles of 95 °C for 1 min, 48 °C for 1 min, 65 °C for 4 min with ramp of 0.3/s to 65 °C; one cycle of 65 °C for 5 min. For psbA-trnH, rps16, rpoCI, ndhF-rpl32 and trnL-F, the $25-\mu L$ reaction mix consisted of 16.1 μ L of H₂O, 2.5 μ L of *Taq* buffer, 2.5 μ L of dNTP mix (10 mM concentration for each nucleotide), 0.75 μ L of each primer (10 μ M concentration), 1.25 μ L of MgCl₂ (50 mM concentration), $0.125 \mu L$ of *Taq* polymerase (0.625) units), and $1 \mu L$ of DNA template. In some cases of nonamplification, $2 \mu L$ of DNA template was used (with the volume of H2O adjusted accordingly. Cleaned PCR products were sequenced by using ABI capillary sequencers (Applied Biosystems) at the University of Edinburgh and Northwoods DNA. Sequences were assembled by using Sequencher v4.5 (Gene Codes) and aligned manually, which was unproblematic given low sequence divergence.

Bayesian analysis was performed by using MrBayes 3.1.1 (4) with 5,000,000 generations of four simultaneous MCMC chains, sampling one tree every 10,000 generations. ModelTest 3.7 (5) was used to select the best-fitting substitution model for each plastid region. Phylogenetic trees were rooted by using outgroup sequences from *Zygia*, which is shown to be most closely related to *Inga* in phylogenetic analyses by using multiple genera from tribe Ingeae.

We ran initial analyses using all accessions from both Peru and Panama. Because in all cases accessions of species found in both Peru and Panama were resolved as monophyletic, or nearly so, we reduced each to a single accession so as not to bias analyses attempting to detect phylogenetic signal.

For subsequent ecological analyses that involved phylogenies, we randomly selected 100 or 200 after burn-in phylogenetic trees from the Bayesian phylogenetic analysis. All trees were made ultrametric before subsequent analyses by using nonparametric rate smoothing (6) in the APE package (7) of the R statistical environment (R Core Development Team 2009). All analyses were also conducted by using a set of 173 equally parsimonious trees and gave equivalent results.

Relationships Among Defense Traits. We evaluated relationships between expansion rate, chlorophyll content, and ant visitation for all species by using conventional least-squares linear regression and linear models with PICs and forcing the intercept through zero (8). For analyses that involved all species, trait values were averaged between Peru and Panama for shared species. Analyses were conducted in the R statistical environment (R Core Development Team 2009). We report *r*² values adjusted for sample size and parameter number. PICs were obtained by using the APE package in R. For the ant visitation character, differences among species in visitation rates were compared by using data that were normalized for each site because ant abundance was 2 times higher in Peru.

Chemical Defense Similarity. We evaluated chemical dissimilarity (or distance) between species as the total number of compounds for which they differed in presence/absence state, standardized by the maximum value for this metric across all species pairs (thus, distance varies from 0 to 1). We first calculated dissimilarity separately for phenolics and saponins. To obtain the total

chemical distance between species, we combined the phenolics and saponins data and upweighted saponins such that the maximum distance for saponins would be equal to the maximum distance for phenolics. For BCI Inga, we also calculated the chemical distance for nonprotein amino acid composition. We evaluated the correlation between chemical distances and phylogenetic distance (across the 200 Bayesian trees) by using Mantel tests (9).

We constructed a dendrogram for the chemical composition data by using hierarchical clustering (10) of the equally weighted phenolics and saponins data. We assessed support for the dendrogram by using multiscale bootstrapping and calculating the approximately unbiased *P* values for nodes with the pvclust package (11) in R.

Evolutionary Lability in Escape vs. Defense and Ant Visitation. Because the above analyses indicated that expansion rate and chlorophyll content covaried strongly, we used a PCA to derive independent axes of defense trait variation for variables for which we had continuous data (expansion rate, chlorophyll content, and ant visitation). The PCA produced two axes with eigenvalues >1 . The first axis was highly correlated with expansion rate $(r = -0.71)$ and chlorophyll content $(r = 0.70)$, whereas the second axis was highly correlated with ant visitation $(r = 0.98)$.

We assessed whether there was significant phylogenetic signal for these two defense axes by determining whether Blomberg's *K* (12) was significantly different from 0, based on 999 randomizations of the data (across 200 Bayesian trees; using the R package Picante; http://picante.r-forge.r-project.org).

We were lacking expansion rate or chlorophyll content data for 1/3 of the species in Peru. To include these species in analyses, we derived a binary characterization, which represented the two extremes of the developmental defense syndrome. For species with chlorophyll and expansion data, species were classified as escape vs. defense (13) based on where the species were placed on a plot of expansion rate by chlorophyll content (see Fig. 2). For most of the remaining species where data were incomplete, T.A.K. and P.D.C. classified them as escape or defense by using their extensive field experience with visually estimating chlorophyll content [\(Table S3\)](http://www.pnas.org/cgi/data/0904786106/DCSupplemental/Supplemental_PDF#nameddest=ST3).

We assessed phylogenetic signal for this escape/defense character by optimizing the character under a maximum parsimony criterion onto the 50% majority rule Bayesian consensus tree by using Mesquite version 2.01 [\(Fig. S4](http://www.pnas.org/cgi/data/0904786106/DCSupplemental/Supplemental_PDF#nameddest=SF4) and ref. 14). To account for topological uncertainty, the procedure ''Trace over Trees'' was used to summarize ancestral state reconstructions >100 Bayesian trees sampled at stationarity. To test whether phylogenetic distribution of escape/defense was significantly different from random the number of parsimony steps in these characters was measured across 100 Bayesian trees sampled at stationarity was compared with: (*i*) the number of steps in the same characters optimized on to 1,000 random trees produced in MacClade 4.08 (15) and (*ii*) the number of steps in the same characters when the states were randomized among terminal taxa using the ''reshuffle character'' option in Mesquite (14). We used a similar approach to visualize the evolution of a binary character repre-senting ant visitation rates (high vs. low visitation; [Fig. S4\)](http://www.pnas.org/cgi/data/0904786106/DCSupplemental/Supplemental_PDF#nameddest=SF4).

We also determined whether developmental and ant defense axes (axes 1 and 2, respectively, from PCA analyses above) were orthogonal to chemical defenses. We first obtained distance matrices for these axes by calculating the Euclidean distance between species for each axis. We then used Mantel tests to assess whether there was a significant correlation between each distance matrix and the chemical distance matrix.

Community Phylogenetic and Defense Trait Dispersion. We evaluated the phylogenetic and defense trait structure of Inga communities by using the inverse of the NRI and NTI of Webb (16) and as calculated following Kembel and Hubbell (17). We conducted analyses separately for Panama and Peru and only used species from each location when randomly drawing species for null communities. We shuffled tip labels in the phylogeny (18) to generate null communities for calculating the normalized metrics NRI and NTI. There was no significant phylogenetic signal for abundance in Peru or Panama (across 200 Bayesian trees; Peru: $\bar{K} = 0.64$, $\bar{P} = 0.36$; Panama: $\bar{K} = 0.96$, $\bar{P} = 0.12$), although lack of significance may be caused by low sample size, particularly in Panama.

We calculated a defense distance matrix between species separately for Peru and Panama by averaging the distance matrices for the different chemical classes above (the nonprotein amino acid matrix being present for Panama only) with an ant visitation rate distance matrix (absolute distance between species in ant visitation rate, standardized by the maximum value) and an escape vs. defense distance matrix (where matrix cells have a binary state of 1 if the two species differ in this classification and 0 if they are the same). Thus, the different chemical defense classes, ant visitation rate, and escape vs. defense were all weighted equally. We obtained a bio-neighbor joining tree from the total defense distance matrix and treated this as a phylogenetic tree to evaluate whether there was a signal for abundance in the defense data. There was not (Peru: \bar{K} = 0.12, $P = 0.85$; Panama: $K = 0.59$, $P = 0.62$), and we therefore shuffled species labels in the defense distance matrix to generate null communities for defense trait structure analyses.

We evaluated whether the distribution of NRI and NTI values for communities were significantly different from zero by using *t* tests following Kembel and Hubbell (17). All phylogenetic and defense trait structure analyses were conducted by using functions in the Picante and APE packages in R (http://picante. r-forge.r-project.org).

We also conducted defense community structure analyses by using the first two axes from the PCA above to represent developmental and ant defense trait variation. Specifically, we averaged the chemical distance matrices with a distance matrix derived from the Euclidean distance between species along the first two principal component axes. This necessarily resulted in a loss of sample size, with regard to species, for Peru because many species were lacking expansion and chlorophyll data. This analysis gave results in the same direction as the above analyses $($ Panama: $\bar{N}RI = 0.48, t = 8.74, P < 0.00001; \bar{N}TI = 0.59, t =$ $10.53, P < 0.00001$; Peru: \bar{N} RI = 0.08, $t = 0.34, P = 0.370$; \bar{N} TI = $0.24, t = 1.36, P = 0.092$.

To determine whether other traits, not related to herbivore defense, showed a similar pattern of community structure, we obtained data for each species on the presence of wings on the leaf rachis and the number of leaflets per leaf [\(Table S3\)](http://www.pnas.org/cgi/data/0904786106/DCSupplemental/Supplemental_PDF#nameddest=ST3). We created a distance matrix for calculation of NRI and NTI values by averaging distance matrices for the first two axes from a PCA of these characters.

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Fig. S1. Important shikimic acid/phenylpropanoid/flavonoid pathway intermediates and branch points in *Inga*. Only a subset of the biosynthetic steps are shown. The acronyms refer to biosynthetic precursors and biosynthetic intermediates. The precursors include **1**, erythrose-4-phosphate phosphoenolpyruvate; **2**, dehydroquinic acid; **3**, dehydroshikimic acid; **4**, L-arogenate, and **5**, phenylalanine. The biosynthetic intermediates are **15**, naringenin and **16**, dihydroquercetin. The structures of the intermediate adducts, the compounds that do not accumulate to a high level in vivo as monomers but that are components of important defense metabolites, include: **7**, tyramine; **8**, quinic acid; **10**, gallic acid; **11**, cinnamic acid, and **14**, coumaric acid. The 18 end-products, the major defense metabolites that accumulate to a high level in vivo, are: **6**, tyrosine; **9**, quinic acid gallate; **12**, tyrosine gallate; **13**, tyramine gallate; **17**, dihydromyricetin; **18**, catechin/epicatechin (c/e); **19**, gallocatechin/galloepicatechin (gc/ge); **20**, mixed c/e-gc/ge polymer; **21**, c/e polymer; **22**, gc/ge polymer; **23**, 3-*O*-galloyl c/e; **24**, 3-O-pyrano-galloyl/cinnamoyl c/e; 25, 3-O-galloyl gc/ge; 26, 3-O-coumaroyl gc/ge; 27 and 28, c/e polymer variably substituted at the 3-O-position; 29 and 30. gc/ge polymer variably substituted at the 3-*O*- position. Six of the 18 listed end-products, **18**, **19**, and **23**-**26**, are also the monomeric building blocks of the indicated polymers. The other 12 end-products correspond to the 12 classes of phenolics referred to in *Results*. After Xie and Dixon (19).

Fig. S2. The rate of expansion of young leaves expressed as the percentage increase in area per day (% per day) versus the number of ants visiting extrafloral nectaries on young leaves of *Inga* species. There was no significant relationship for either Panama (solid line, $r^2 = 0.11$, $P = 0.75$, $n = 11$) or Peru (dotted line, $r^2 = 0.069$, $P = 0.24$, $n = 22$).

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THE TENN CHANGED AND THE USE OF THE **Fig. S3.** Average chemical distance (weighting phenolics and saponins equally) between species by phylogenetic distance between species for one randomly selected Bayesian tree. The line represents the best-fit linear regression (*y* = 0.28 + 0.086*x*), while significance and fit of the relationship was evaluated by using a Mantel test (for this tree: $r = 0.12$, $P = 0.031$).

Fig. S4. Developmental and ant defense mapped onto *Inga* phylogeny. (*A*) Binary character of defense (white) and escape (black) optimized by using parsimony onto 50% majority rule Bayesian consensus tree (gray shading indicates equivocal state). Numbers adjacent to nodes are posterior probability values. To account for topological uncertainty, ancestral state reconstruction was summarized for >100 Bayesian trees sampled at stationarity, and along each branch the thickness of the line representing each state is in proportion to the number of trees in which that state is reconstructed. Low phylogenetic signal is indicated by 8–11 observed parsimony steps, which is well within the frequency distribution of the number of steps when states are randomized across terminal taxa (''reshuffle character'' option in Mesquite 2.01) (14). Furthermore, the 8–11 observed transformations lie within the frequency distribution of transformations when mapped onto 1,000 random trees generated by MacClade 4.08 (15). (*B*) Binary character of low (white) and high (black) ant visitation (gray shading indicates equivocal state) optimized using parsimony onto 50% majority rule Bayesian consensus tree. Numbers adjacent to nodes are posterior probability values. To account for topological uncertainty, ancestral state reconstruction was summarized for >100 Bayesian trees sampled at stationarity, and along each branch the thickness of the line representing each state is in proportion to the number of trees in which that state is reconstructed. Low phylogenetic signal is indicated by 10–13 observed parsimony steps, which is well within the frequency distribution of transformations when randomized across terminal taxa (reshuffle character option in Mesquite 2.01) (14). Furthermore, the 10–13 observed transformations lie within the frequency distribution of transformations when mapped onto 1,000 random trees generated by MacClade 4.08 (15).

Fig. S5. The protocol used to resolve *Inga* young leaf extracts into chemically distinct fractions. The term "marc" refers to the insoluble cell walls. ODS refers to liquid chromatography on an octadecyl silane (or reversed-phase silica) column. AA, amino acids; OA, organic acids.

Table S1. Distributions of *Inga* **chemotypes**

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Each chemotype is a distinct combination of metabolites. Shown are the major phenolic/saponin chemotypes for all 37 study species (Peru and Panama) and the major amino acid chemotypes for 11 study species from Panama.

Table S2. Gallocatechin/galloepicatechin gallate structures (cf. [Fig. S1\)](http://www.pnas.org/cgi/data//DCSupplemental/Supplemental_PDF#nameddest=SF1) inferred from MS analysis of *Cojoba arboreab* **phenolic extracts and shared among 11 of the 37** *Inga* **study species**

The nominal mass refers to the actual mass, in atomic mass units, of the polymer rounded to the nearest integer. Each unit of the polymer contains either one gallocatechin or one galloepicatechin. These are identical in molecular formula and differ in stereochemistry. Therefore, the degree of polymerization is noted as gallocatechin-galloepicatechin no. The number of gallate esters is noted as gallate no. The minimum esterification with gallate is zero on the entire polymer (e.g. nominal mass 1,522, a tetramer with no gallate). The maximum esterification is one for every gallocatechin/galloepicatechin unit of the polymer (e.g. nominal mass 1,370, a trimer with three gallates). Because the table is ordered by nominal mass and because of the variation in the number of gallates per polymer, gallocatechin-galloepicatechin no. is not in numerical order. Polymers at masses in excess of the highest shown, 1,674, exist and are minor components. In the species of *Inga* characterized to date, the gallocatechin/galloepicatechins and gallic acids polymerize such that a dimer could, in principle, have eight or more forms of identical mass. Although not indicated here, we observe a subset of the possible forms of dimers, trimers, tetramers, and pentamers. These forms are distinguished as having identical masses but often distinct chromatographic behavior; they have not been fully characterized.

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Developmental strategies of escape or defense were assigned based on chlorophyll and expansion (see Fig. 2). Because ant visitation was different between sites, we characterized the numbers of ants at nectaries as high (H) if they were above the site average or low (L) if they were below the site average. The presence or absence of wings on the rachis and the number of leaflets per leaf were determined for saplings in the field.

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Table S4. Collection details and GenBank accession numbers of material used for phylogenetic study

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Duke, Duke University, Durham, NC; K, Royal Botanic Gardens, Kew, United Kingdom; STRI, Smithsonian Tropical Research Institute, Balboa, Panama; MOL, Universidad Nacional Agraria, La Molina,
Lima, Peru. Dash indicates no

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