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Convergence in defense syndromes of young leaves in tropical rainforests

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Abstract

In tropical forests, the majority of damage by herbivores or pathogens occurs on young leaves, yet the patterns of damage and the factors that influence them are poorly known. By measuring damage throughout leaf development and maturation for five species in a Panamanian forest, we showed that leaf toughening, which only occurs over a few days once the leaf is fully expanded, is the main factor decreasing damage in mature leaves. Although rates of damage to young leaves are, on average, orders of magnitude greater than on mature leaves, there is significant interspecific variation in young leaf defenses and in damage rates. In a survey of 55 species of shade-tolerant plants, we found that each species only invested in a subset of the potential defensive mechanisms for young leaves. We measured rates of young leaf expansion, nitrogen content, delayed chloroplast development, synchrony of leaf production and rates of damage in the field. On a subset of 24 species, we also measured phenolic compounds, checked for the presence of saponins and alkaloids, and conducted bioassays using lepidopteran, coleopteran and orthopteran herbivores and four fungal pathogens to test for toxicity of young leaf extracts. Certain combinations of traits repeatedly co-occurred across unrelated species suggesting convergent evolution. We argue that selection has repeatedly led to tradeoffs among defenses such that species fall along an escape/defense continuum. At one extreme are species with a 'defense' strategy, which includes effective chemical defense, slow leaf expansion, normal greening and low rates of damage (less than 20% of the leaf area lost). At the other extreme are 'escape' species which have ineffective chemical defenses and, as a consequence, have high rates of leaf damage, >60% of leaf area lost during expansion. In partial compensation for ineffective chemical defense, these species have very rapid leaf expansion (doubling in area every day) which minimizes the window of vulnerability, delayed chlor-

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oplast development (white young leaves) which contain fewer resources, and synchronous leaf production to satiate herbivores. Thus, interspecific variation in young leaf damage rates is explained by differences in defense combinations along this escape/defense continuum. Because apparently beneficial traits such as effective chemical defense and rapid leaf expansion do not occur in the same species, we suggest that physiological constraints limit the defense combinations of any one species to a restricted subset of those observed. However, the defense and escape strategies do not represent different tradeoffs that have equal fitness, as species with the escape syndrome suffer much higher rates of damage. We hypothesize that the escape syndrome arose over evolutionary time among plants that failed to evolve effective secondary metabolites while herbivores succeeded in evolving adaptations to the chemistry of their host plant. Hence the defense syndrome should provide the greatest fitness, whereas the escape syndrome minimizes damage given the failure of the plant's secondary metabolites to provide protection.

Keywords: Herbivory; Rainforest; Barro Colorado Island; Young leaves; Phenolics; Leaf expansion; Pathogens; Bioassays; Toughness; Delayed greening; *Inga*

1. Introduction

Plants and herbivores comprise more than 50% of the organisms on Earth, and their interactions have profound implications for both ecological and evolutionary processes. These interactions are particularly important in the tropics, where strong reciprocal selection by plants and herbivores has led to both higher rates of herbivory and greater investments in defenses for tropical as compared to temperate trees (Levin and York, 1978; Coley and Aide, 1991; Marquis and Braker, 1994; Basset, 1994). In addition, for shade-tolerant tropical plants, the majority of damage occurs during the short window when leaves are young and expanding (Marquis, 1991; Marquis and Braker, 1994; Coley and Kursar, 1996). For example, daily rates of leaf consumption on young leaves are $>$20 times higher than on mature leaves (Coley, 1983; Coley and Kursar, 1996; Coley and Barone, 1996). This means that although leaves live for several years, $>$70% of the lifetime damage occurs during the few weeks that leaves are expanding (Coley and Kursar, 1996). Because shade-tolerant species dominate tropical rainforests in terms of numbers of species and individuals, as well as plant biomass (Hubbell and Foster, 1986; Whitmore, 1989; Welden et al., 1991; Lieberman et al., 1995), the interactions between their young leaves and herbivores may also dominate trophic dynamics. Thus, although young leaves are ephemeral, they are key for understanding the evolutionary ecology of plant/herbivore interactions in the humid tropics.

Although young leaves of tropical species suffer high rates of herbivory, they exhibit an even greater diversity of defenses than mature leaves (Coley and Barone, 1996). They can protect themselves with a battery of secondary metabolites, often having higher concentrations as well as compounds not found in the mature leaves (Kursar et al., 1999; Coley and Aide, 1991). Physical defenses, such as trichomes, are common, as are extrafloral nectaries which attract ants as bodyguards (Bentley,

1977; Schupp and Feener, 1991). Young leaves can also expand rapidly, shortening the period of greatest vulnerability to herbivores (McKey, 1979; Aide and Londoño, 1989; Ernest, 1989; Kursar and Coley, 1991), and leaves can be produced synchronously to satiate specialist herbivores (Lieberman and Lieberman, 1984; Aide, 1993). Many tropical species have young leaves that are white, pink or very light green, a dramatic visual display that has captured the imagination of naturalists for over a century (Richards, 1952; Smith, 1909). This phenomenon of delayed greening or delayed chloroplast development is a feature that lowers the nitrogen and energy content of young leaves, thereby reducing the negative impacts of a given amount of herbivory (Kursar and Coley, 1991, 1992a). Surprisingly, despite this diverse array of defense options and the high rates of damage to young leaves, a given species only invests in a subset of defenses (Coley and Kursar, 1996).

Here we report results on herbivory and defenses for young leaves from a community survey of shade-tolerant plants in a lowland rainforest in Panama. We ask why rates of herbivory are so high on young leaves, and why only certain combinations of defensive traits are found in nature. To answer the first question, we provide evidence that young leaves have high protein contents and low toughness; two unavoidable consequences of leaf growth that make them particularly attractive to herbivores. To answer the second question, we show that, although young leaves have a variety of defensive options, including chemical, physical and developmental traits, not all defenses are physiologically compatible. Thus, we suggest that selection has repeatedly led to tradeoffs among defenses, such that species fall along an escape/defense continuum. At one extreme are species that are well defended chemically, and at the other, are species that have put all resources into rapid growth so as to minimize the window of expansion when they are vulnerable to herbivores. Finally, we suggest that the effectiveness of a species' chemical defenses may determine whether selection has favored an evolutionary trajectory of defense or escape.

2. Methods

2.1. Study site and species

The study was conducted on Barro Colorado Island (BCI, 9° 09′ N, 79° 51′ W) in the Republic of Panamá, a field site administered by the Smithsonian Tropical Research Institute (STRI). The forest is moist lowland forest (Holdridge et al., 1971; Leigh et al., 1982; Leigh, 1997; Croat, 1978) and receives 2600 mm of rain during an 8-month rainy season (Windsor, 1990). We collected data on herbivory and leaf traits for 55 species of plants from 1996 until 2001. All species are shade-tolerant and include trees, shrubs and lianas and represent 39 genera and 24 families. In order to make phylogenetically controlled comparisons, we also examined trends between herbivory and leaf traits for *Inga*, a speciose genus of trees in the Fabaceae, subfamily Mimosoideae (Appendix 1a). All data were collected on individual plants between 0.5–2 m tall growing in the shaded understory.

2.2. Herbivory

Young leaves were marked as they emerged from the bud. So as not to damage the expanding leaf, we placed color-coded telephone wire on a mature leaf near the flush, and identified young leaves by the number of nodes distal to the marked leaf. At the end of leaf expansion, between 2–8 weeks depending on the species, leaf and hole areas were measured with a plastic grid to determine the percent of leaf area eaten while young. Our marking technique also allowed us to determine if leaves were missing. Missing leaves were scored as 100% damage. Plants or leaves that were obviously damaged by falling debris were not included.

In addition, we obtained more detailed information on herbivory for four shade-tolerant species in the understory and one light-demanding specialist in a gap (Appendix 1b). We censused herbivory every 2 to 5 days for hundreds of young leaves throughout leaf expansion and for 60 days after the leaves reached full size (see Fig. 1 for sample sizes). Data were collected in the wet seasons of 1982 to 1985.

2.3. Leaf traits

2.3.1. Toughness

Leaf toughness was measured by using a Chatillon pressure gauge (Chatillon Inc., Chicago). The leaf was clamped between two Plexiglas plates, each drilled with a 4-mm diameter hole. The number of grams of weight necessary to punch a 3-mm diameter rod through the leaf gives an index of toughness. The following equation converts the toughness index (g) into force per area, 1.0 g = 1.38 kPa.

2.3.2. Nitrogen

Young expanding leaves at 50–70% of full size were collected for measurements of nitrogen. Nitrogen content was determined on dried leaf tissue using a 2400 CHN Rapid Analyzer (Perkin-Elmer, Norwalk CT) at the University of Utah.

2.3.3. Chlorophyll

Many species of tropical plants delay chloroplast development until the leaf is full size (Kursar and Coley, 1992a,b,c). Chlorophyll content provides an excellent measure of chloroplast development, as chlorophyll is highly correlated with the amount of rubisco, light harvesting proteins and photosynthetic capacity of leaves (Baker and Hardwick, 1973; Kursar and Coley, 1992a). For chlorophyll analysis, young leaves (between 50–70% full size) were placed in ziploc bags to prevent desiccation and measured within a few hours of collection. Fresh leaves were extracted in 100% acetone, centrifuged for 10 min, adjusted to 90% acetone and absorbances measured at 647 and 664 nm and corrected for light-scattering by subtracting absorption at 720 nm. Chlorophyll concentrations were determined using the equation of Jeffrey and Humphrey (1975). In all spectrophotometric assays, concentration was adjusted such that absorbance was between 0.10 and 1.0.

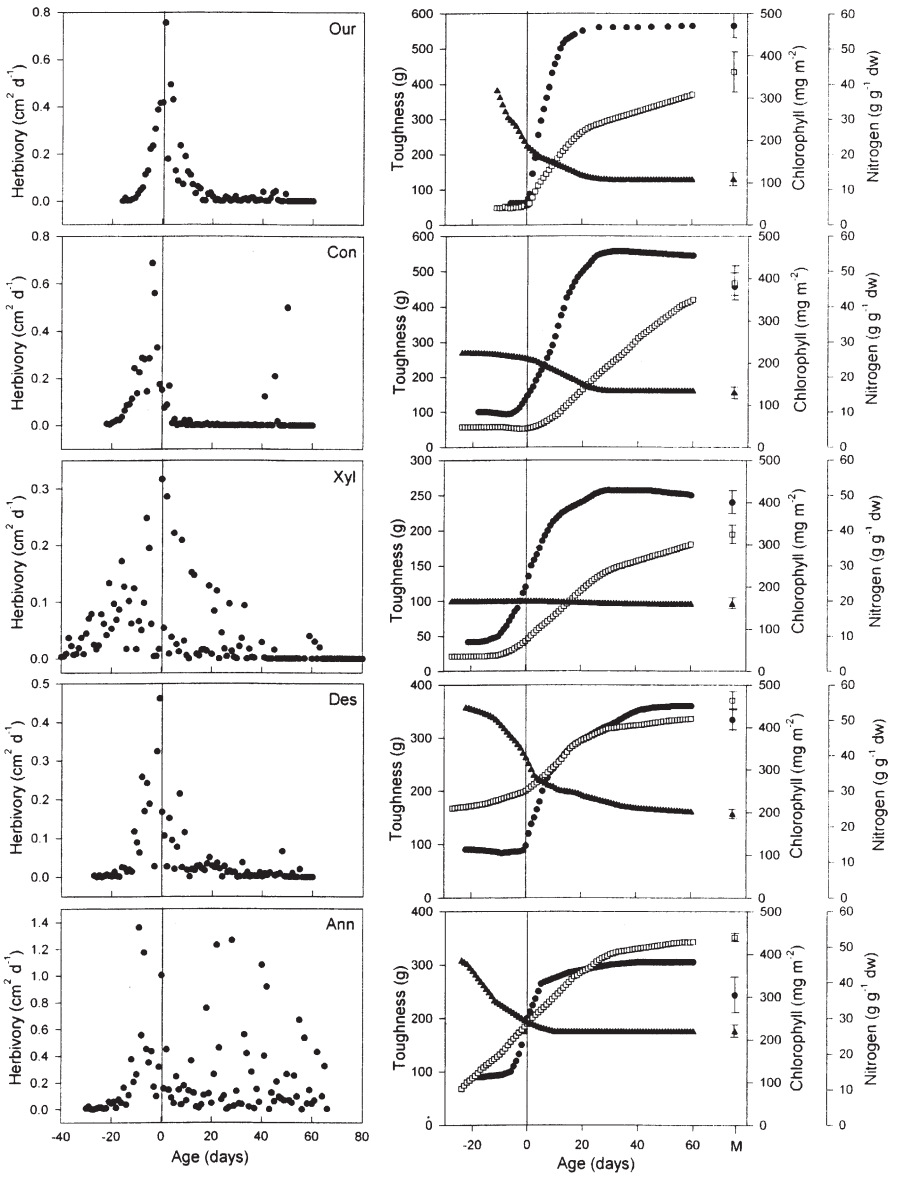

Fig. 1. Rate of leaf damage, leaf toughness, nitrogen and chlorophyll content throughout expansion for five species in Panama (Appendix 1a). Age zero is the day at which leaves reach full size. Negative ages are during expansion of young leaves. Because leaves expand at different rates, species differ in the number of days of data during expansion. Rates of leaf damage, reported as $cm^2\ d^{-1}$, were measured in the wet seasons of 1982–1985. Sample sizes of plants and leaves are as follows: *Ouratea* (80 plants, 436 leaves), *Connarus* (88 plants, 618 leaves), *Xylopia* (41 plants 493 leaves), *Desmopsis* (35 plants, 482 leaves), and *Annona* (19 plants, 354 leaves). Values for toughness (solid circles), nitrogen (solid triangles) and chlorophyll (open squares) are the smoothed averages of measurements on >100 leaves. 'M' indicates the mean +/− standard deviation for randomly collected mature leaves.

2.3.4. Secondary metabolites

Total phenolics were determined after reacting Folin-Ciocalteu reagent (Sigma) with a methanolic plant extract (Waterman and Mole, 1994). Freeze-dried leaves (0.25 g) were pulverized, extracted with 40 mL of 90% methanol, homogenized in a Polytron for 2 min, allowed to extract for 1–12 h at 5 °C, centrifuged at 10 000 g for 10 min at 5 °C, the supernatant dried under vacuum, redissolved in 20 mL of 50% methanol/water (v/v), and extracted with 20 mL of hexane. To assay, 0.12 mL of the methanol/water fraction was added to 0.88 mL of water, and then 2 mL of Folin and Ciocalteu phenol reagent (Sigma), incubated for 3 min, 2.0 mL of 0.50 M Na_2CO_3 was added, incubated for 60 min and the absorbance read at 725 nm.

Astringency was determined from hemoglobin precipitated by the plant extract (Waterman and Mole, 1994). We extracted metabolites from fresh young leaves between 40–80% of full size. Leaves were homogenized in 10 mL methanol per gram fresh weight of leaf using a Polytron (Brinkmann Instruments; Torti et al., 1995) and centrifuged for 2 min at 500 g. The pellet was extracted with diethylether (8:1; mL:fresh weight), centrifuged and the pellet extracted a final time with water (6:1; mL:fresh weight) and centrifuged. The three extracts were combined, lyophilized to dryness (Speed-Vac, Thermo Savant, Holbrook, NY) and stored at −50 °C until use. For assays, extracts were redissolved in diethylether (1.5 mL per extract from 1.5 g fresh weight of leaf), centrifuged for 10 min at 15 600 g and the pellet was dissolved in 1.5 mL methanol:water (1:1; v/v). The ether and methanol extracts were combined.

The above preparations were used in all herbivore and fungal bioassays, as well as for the astringency assay. For astringency, the ether was allowed to evaporate, 0.03 mL of extract was combined with 0.37 mL of water, and this was mixed, very gently to avoid protein denaturation, with 0.6 mL of hemoglobin solution (Sigma H-2625, 2.2 mg mL^{-1} in 0.10 M $K/NaPO_4$ buffer, pH 6.0). After a 30 min incubation at room temperature, the sample was centrifuged at 15 600 g for 10 min at 5 °C, applied to a 10 mL column of Sephadex G-25-80 (Pharmacia), eluted with water, fractions 5 to 7 containing the hemoglobin were combined, and absorbance at 520 nm determined. In both assays, tannic acid (Mallinkrodt Lot 1764 KCNT) was used as a standard.

Alkaloids were detected in methanolic extracts using Dragendorf's reagent following TLC on silica gel G with methanol:NH_4OH (200:1; v/v) and saponins were detected in hexane extracts using $SbCl_3$ reagent following TLC on silica gel G with chloroform:acetone (4:1; v/v).

2.4. Leaf expansion

We measured the rate of leaf expansion, an important trait that determines how long young leaves are vulnerable to herbivores. Leaves at approximately 15% of full size were marked with telephone wire, and their area measured every 1–4 days (depending on the species) until they reached full size. The increase in leaf size with time was exponential for areas between 15 and 80% of the final size. We calculated

the daily percent increase in size during the expansion phase using the following equation:

$$\text{Expansion rate as percent per day} = 100*[e^{(\ln(\text{area2}/\text{area1})/\text{time})}-1]$$

where 'area1' and 'area2' are leaf areas at two different measurements and 'time' equals the number of days between measurements. Values of 100% per day indicate that the leaves doubled in size daily. Species that expanded more slowly had lower values of expansion. Expansion rate shows very little influence of light environment.

2.5. Bioassays

2.5.1. Experimental design

In order to assess the extent of chemical defense, we tested leaf extracts for toxic or inhibitory affects against herbivores and fungi. Because we were interested in testing the idea that species with rapid leaf expansion invest less in chemical defenses, we used a paired experimental design (Appendix 1c). Six pairs included two species from the same genus or family, one with rapid leaf expansion and one with slow. These pairs control for phylogenetic and chemical similarity and provide a strong test of the correlation between expansion and chemical defense. An additional six pairs included unrelated species, each pair containing an extremely fast and slow expander. This allowed us to sample a larger range of expansion rates than was possible with phylogenetically constrained comparisons.

2.5.2. Extracts

For assays, we extracted metabolites from fresh young leaves, stored the freeze-dried extracts at −50 °C, and then redissolved them as described above. The ether and methanol extracts were combined and either painted on leaves for herbivore trials or incorporated into medium for fungal growth assays.

2.5.3. Insects

We chose three species of insects for feeding trials, representing three important orders of herbivores: *Spodoptera exigua* (Lepidoptera), *Schistocerca americana* (Orthoptera) and *Chelimorpha alternans* (Coleoptera). *Spodoptera* eggs were obtained from the Western Cotton Research Laboratory (Phoenix, AZ) and raised on a lima bean diet (Guerra and Bhuiya, 1977) including 5 mL of COAX, a feeding stimulant, per liter of diet, with agar from ICN Biochemicals, ascorbic acid from VWR, and all other reagents from Sigma. For bioassays, organically grown romaine lettuce leaves were purchased in a local store and rinsed in distilled water. A 1-cm diameter disk (0.785 cm^2) was painted with extract such that the dry weight of extract per area of lettuce leaf equaled 100% of that found in the species under study. We placed two disks, one for each pair of species, and one 4th or 5th instar larva in a petri dish with moistened filter paper and evaluated consumption 1–3 h later when about 50–60% of the total leaf area had been consumed. *Schistocerca* were maintained on wheat in a colony at the University of Utah. We used animals 1.5–3.0 cm long in bioassays. Wheat leaves, 2.4 cm^2 in area, from 1 to 3-week-old greenhouse-

grown seedlings, were painted with extract such that the dry weight of extract per area of wheat leaf equaled 100% of that found in the species under study. We placed two pieces of wheat leaf, one for each pair of species, and one grasshopper in a plastic box with moistened filter paper and evaluated consumption 1–24 h later when about 50–60% of the total leaf area had been consumed. For *Chelimorpha*, we painted extracts on to a 1-cm^2 disk of the preferred host plant, sweet potato. Extracts were painted at amounts equivalent to 5% of the concentration of natural leaves. Individual herbivores were presented with two leaf disks, one from the fast- and slow-expanding species of each pair, and the area of leaf tissue eaten was measured after 24 h. Each experiment was repeated with >25 individual herbivores. In all three experiments, the difference in leaf area eaten was calculated to give a preference score (fast-slow).

2.5.4. Fungi

Four species of fungi isolated on BCI by Dr. Gregory Gilbert were used in growth assays. We used two isolates of *Pestalotiopsis*, a widespread genus of leaf-spotting pathogen and two opportunistic fungi that also attack leaves, *Fusarium* and *Penicillium*. Fungi were cultured on malt extract agar (5 g malt extract, 3.75 g agar (both from Difco) and 250 mL water, autoclaved for 30 min). For bioassays, plant extracts were resolubilized in methanol and ether (as described above) and a range of concentrations were added to sterile 96-well plates. Wells contained extract from 0, 0.75, 1.5, 3.0, 6.0, 12, 24 or 48mg fresh weight of leaf. Plates were placed in a laminar flow hood to allow solvents to evaporate before medium containing the test fungus was added. One week before bioassays, agar sections from fungal stock plates were used to inoculate 40 mL sterile malt extract broth (ME, 0.32 g malt extract, autoclaved for 30 min) which was cultured at 30 °C on a rotary shaker. On the day of the assay, 0.5 mL was transferred to 10 mL ME broth and the mass of fungal hyphae broken down into a uniform suspension using a Polytron homogenizer (30 K r.p.m., 45 s). Immediately, 75 μl of the fungal suspension was added to each of the 96 wells. Ten μl of Nystal (nystatin 100 000 IU/mL, Halgam, S.A., Panama) was used as a positive control. Other controls included fungi without plant extracts, as well as plant extracts without fungi. Experiments were repeated six times.

Growth was scored after 5 days at 30 °C and compared to controls where fungi grew with only medium and solvent. Because all fungal species were dark colored, growth was scored visually. The extract concentration that inhibited growth to 50% of the control was used as a index of growth inhibition (GI_{50}). For each pair, we obtained an inhibition index which was the difference between the GI_{50} values (fast-slow). Thus a value means the extract from the slow-expanding species was more inhibitory.

For each herbivore or fungal species, we analyzed the 12 preference and inhibition scores (one from each pair in Appendix 1c) using a paired t-test ($n = 12$).

3. Results

Rates of damage during young leaf expansion were markedly higher than for mature leaves (Fig. 1). For the four shade-tolerant species, the damage rate dropped rapidly, over only a few days, as soon as the leaf reached full size and began to toughen. Fig. 1 presents data as the cm^2 of leaf lost per day. Expressing it as the percent of leaf area lost per day also demonstrates dramatic declines with age (Table 1). Damage rates (%/d) for shade species are from 7 to >300 times higher on young leaves. For *Annona*, the gap-requiring species, damage is high for young leaves (Fig. 1, Table 1), but does not decline as sharply for mature leaves. This is a typical pattern for pioneer species which invest little in the chemical defense of their short-lived mature leaves. For *Connarus*, the few events of high herbivory at approximately 40 days (Fig. 1) were due to a lepidopteran stem borer that had originally attacked the tender stem when leaves were young. For all species, rates of damage ($cm^2\ d^{-1}$) were low early in expansion because leaves are small. However, if damage is expressed as a percent of leaf area removed per day, rates were high throughout expansion. Thus, tissue loss to herbivores is concentrated during a short window of leaf expansion but can be extremely high.

The vast majority of the damage to young leaves is done by insect herbivores. *Xylopia* was unusual in having high rates of pathogen damage which accounted for 21% of the leaf loss. In an additional survey, we followed the fate of 240 *Xylopia* leaf flushes which had initiated growth, but remained at the bud stage. In 55% of the cases, the entire bud died because of pathogen attack. For all other species, pathogen damage was low, and >95% of the damage was due to insect herbivores.

The high rates of herbivory to young leaves are correlated with low toughness and high nitrogen content during leaf development (Fig. 1). Although the expansion period ranges from 10 days for *Ouratea* to 40 days for *Xylopia*, all species began to toughen as soon as the leaves reached full size. So, within a few days, leaves had stopped expanding, toughened substantially, and experienced a dramatic reduction

Table 1
Percent of leaf area lost daily to herbivores and pathogens for young (age <=0) and mature leaves (age >10)

Species	Habitat	Expansion (%/d)	Young leaves		Mature leaves	
			Mean	Std	Mean	Std
Ouratea	shade	70	4.29	2.34	0.082	0.019
Connarus	shade	32	3.74	2.88	0.012	0.002
Xylopia	shade	15	0.86	0.56	0.124	0.060
Desmopsis	shade	23	0.46	0.51	0.024	0.001
Annona	light gap	20	0.57	0.55	0.226	0.105

Species are classified as being shade-tolerant or light gap-requiring. Expansion rate is the percent daily increase in size for young leaves. Damage rates are the same data as in Fig. 1, but expressed as a percent of leaf area consumed per day (mean and standard deviation).

in herbivory. During this period, nitrogen content also drops, making leaves less attractive.

The amount of leaf tissue lost to herbivores and pathogens during leaf expansion was generally high, but showed substantial interspecific variation (Fig. 2). Several species lose more than 60% of their leaf area during the few weeks of expansion, while others lose less than 20%. Damage rates are positively correlated with leaf expansion rate ($r^2 = 0.15$, $P < 0.01$, $n = 49$). Although species with rapid leaf expansion have a *shorter* window of vulnerability, they suffered significantly *higher* leaf loss. The relationship is even more marked if we restrict the comparison to a single legume genus, *Inga* ($r^2 = 0.79$, $P < 0.02$, $n = 6$; open triangles in Fig. 2).

The counter-intuitive positive correlation between expansion rate and herbivory suggests that rapid expansion may be correlated with other traits that make the leaf more palatable. One factor may be investment in secondary metabolites. We assessed this using extracts from 12 pairs of plant species (each pair containing one species with fast- and one with slow-expanding young leaves, Appendix 1c). Extracts from fast-expanding species were preferred in feeding trails with Lepidopteran and Coleopteran herbivores and were less inhibitory to growth of all four fungal pathogens (Fig. 3). However, there was no correlation between expansion rate and either total phenolics ($r^2 = 0.02$, $P = 0.50$) or astringency ($r^2 = 0.03$, $P = 0.49$). Furthermore, damage rate was not correlated with either total phenolics ($r^2 = 0.02$, $P = 0.52$) or astringency ($r^2 = 0.02$, $P = 0.56$). This suggests that compounds other than phenolics must be responsible for the enhanced chemical protection of slow-expanding young leaves. Although we did not quantify other classes of secondary metabolites, two slow-expanding species, *Guatteria* and *Heisteria*, had alkaloids and three fast- and one slow-expanding species had saponins.

Rapid leaf expansion is also associated with a delay in chloroplast development. In species with delayed greening, young leaves appear white and photosynthetic

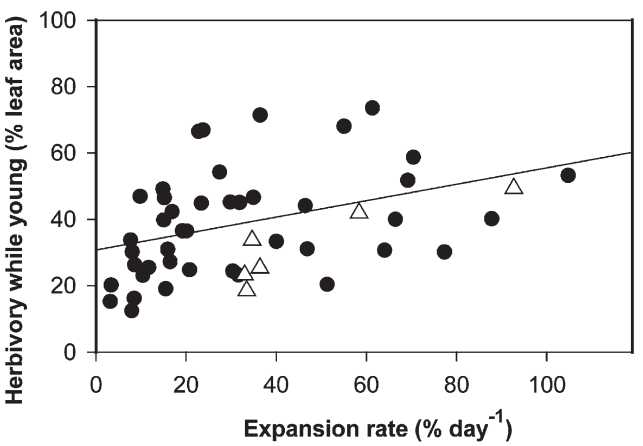

Fig. 2. Leaf expansion rate versus herbivory while leaves are young. The regression line is fitted through all species ($r^2 = 0.15$, $P < 0.01$, $n = 49$, $y = 29.8 + 0.236x$). Open triangles indicate *Inga* species (Appendix 1a), $r^2 = 0.79$, $P < 0.02$, $n = 6$.

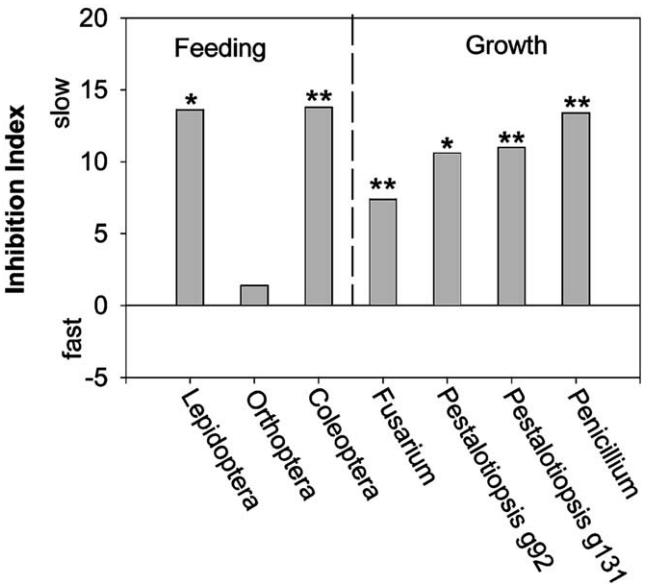

Fig. 3. Bioassays for toxicity of leaf extracts from species with fast- and slow-expanding young leaves. Herbivores used in feeding trials were *Spodoptera exigua* (Lepidoptera), *Schistocerca americana* (Orthoptera) and *Chelimorpha alternans* (Coleoptera). Fungal growth was assessed using two isolates of the leaf spotting pathogen, *Pestalotiopsis*, and two opportunistic pathogens, *Fusarium* and *Penicillium*. The inhibition index is the difference in feeding or growth caused by extracts from the fast-expanding and slow-expanding species of each of 12 pairs (see Appendix 1c). Feeding is the difference in mm^2 eaten (fast-slow) and fungal growth is the difference in GI_{50} (fast-slow). Thus, values >0 indicate greater inhibition by extracts from species with slow leaf expansion. $**P < 0.01$, $*P < 0.05$ (paired t-test, $n = 12$).

capacity and chlorophyll content are extremely low throughout expansion (Fig. 1). It is not until leaves reach full size and are protected by toughness that chloroplast development begins. There is a strong negative relationship between expansion rate and chlorophyll content, a measure of chloroplast development (Fig. 4; $r^2 = 0.57$, $P < 0.001$, $n = 51$). The same pattern is seen if we examine the correlation within the genus *Inga* ($r^2 = 0.35$, $P = 0.07$, $n = 10$). Thus, species with more rapid leaf expansion have significantly lower levels of chlorophyll.

What is the advantage of delayed greening if young leaves are not able to photosynthesize? The advantage is that young leaves of species with delaying greening contain fewer resources that can be lost to herbivores. There is a significant correlation between expansion rate and nitrogen content ($F = 17.1$, $P < 0.001$) and a significant interaction between expansion rate and chlorophyll content ($F = 7.8$, $P < 0.01$) (Fig. 5). Thus, fast expanders have more nitrogen than slow expanders, and for a given expansion rate, young green leaves have more nitrogen than young white leaves. This pattern can also be seen for the three Annonaceae which have similar expansion rates, but *Annona* and *Desmopsis*, with green young leaves have higher nitrogen contents than *Xylopia* with white young leaves (Fig. 1). Also, the correlation

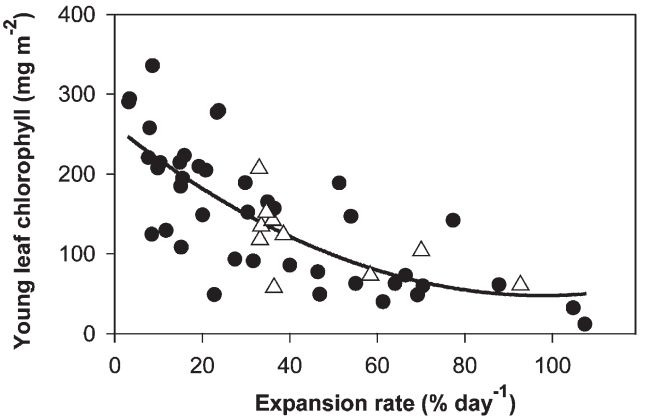

Fig. 4. Leaf expansion rates versus chlorophyll content for young leaves. Open triangles indicate *Inga* species (Appendix 1a). For all species, $r^2 = 0.57$, $P < 0.0001$, $n = 51$ (quadratic regression, $y = 386.9 - 81.03x^{0.3157}$), and for *Inga*, $r^2 = 0.35$, $P = 0.07$, $n = 10$ (linear regression, $y = 179.4 - 1.33x$).

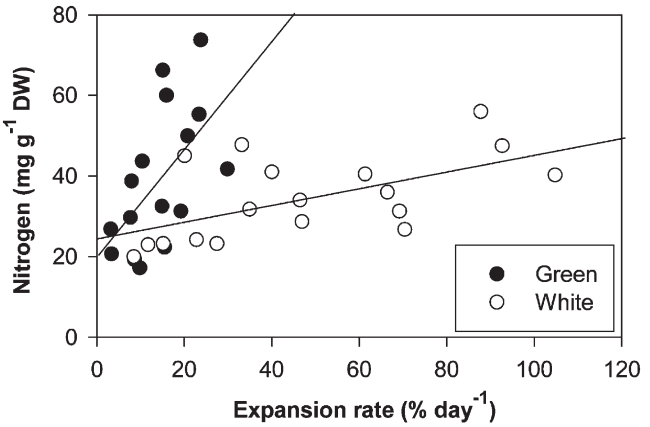

Fig. 5. Nitrogen content of expanding leaves as a function of leaf expansion rate for species with normal and delayed chloroplast development. Solid circles are species which have green young leaves (>175 mg chlorophyll m^{-2}) with normal greening ($r^2 = 0.34$, $P < 0.02$, $n = 16$, $y = 20.1 + 1.34x$) and open circles are species which have white young leaves (<175 mg chlorophyll m^{-2}) with delayed greening ($r^2 = 0.33$, $P < 0.01$, $n = 17$, $y = 24.7 + 0.205x$). There is a significant effect of expansion rate ($F = 17.1$, $P < 0.001$) and a significant interaction between expansion and chlorophyll ($F = 7.8$, $P < 0.01$). Thus, fast expanders have more nitrogen than slow expanders, and for a given expansion rate, green leaves have more nitrogen than white leaves.

between expansion rate and nitrogen is evident for the three white species, where nitrogen is invested in growth rather than photosynthetic function. *Ouratea* has the most rapid rate of leaf expansion (70% d^{-1}) and the highest nitrogen content of young leaves, *Connarus* has medium expansion (32% d^{-1}) and medium nitrogen, and *Xylopia* has low expansion (15% d^{-1}) and low nitrogen (Fig. 1). The advantage

of delayed greening is that white leaves have less nitrogen and energy than green leaves with similar rates of expansion and thus white leaves lose fewer resources for a given amount of herbivory.

4. Discussion

4.1. Dynamic changes in herbivory during leaf development and maturation

This study, as well as others, have repeatedly shown that young leaves of shade-tolerant tropical species suffer much higher rates of damage than mature leaves (Fig. 1 and Table 1; Marquis and Braker, 1994; Coley and Barone, 1996; Dyer and Coley, 2001). However, we are unaware of any other data that have followed damage through leaf development and correlated it with nutritional changes in the leaves. Changes in rates of damage ($cm^2\ d^{-1}$) are very dynamic, increasing exponentially during development and then dropping precipitously within a few days as soon as leaves become tough. Toughness is a particularly effective defense that reduces nutritional value of the leaf and presents mechanical problems for chewing insects (Lowman and Box, 1983; Coley, 1983; Juniper and Southwood, 1986). Mature leaves have abundant cellulose, lignin and other cell wall compounds which makes them tough, however young leaves cannot toughen until leaves have reached full size and cells are no longer expanding. They are thus extremely tender throughout expansion (Fig. 1). Because toughness is such an effective defense, there appears to be strong selection to toughen as soon as physiologically possible. In all the species we have examined, toughening occurs within days of reaching full size, regardless of the length of the expansion period (Fig. 1).

Young leaves also have significantly higher nitrogen per dw (Fig. 1) due to the high protein and low cell wall in growing cells (thin primary cell walls and absence of secondary cell walls). As protein is limiting to most herbivores (Stamp and Casey, 1993), high nitrogen enhances the attractiveness of young leaves to herbivores. Thus, as an unavoidable consequence of growth, young leaves must be tender and nutritious, two traits that contribute to their high rates of herbivory.

Although all five species show similar temporal patterns of herbivory, toughness and nitrogen, deviations are related to life history differences. For example, within the Annonaceae, we sampled three species with different leaf development and shade tolerance. Because *Annona* is a light-demanding gap species, with short-lived and poorly defended mature leaves (Coley, 1983), the difference between herbivory during and after expansion is the least dramatic (Fig. 1 and Table 1). In contrast, *Xylopia* (Annonaceae) is a shade-species with poorly defended young leaves (see ‘escape’ syndrome below), so the changes in herbivory through development are more dramatic. Similar patterns are seen in *Connarus* and *Ouratea*, two unrelated species that are similar to *Xylopia* in being shade-tolerant and having poorly defended young leaves. And finally, the third species in the Annonaceae, *Desmopsis*, has better defended young leaves (see ‘defense’ syndrome below), and shows an intermediate pattern. Thus, mature leaves of shade-tolerant species suffer almost no damage, while

herbivory to young leaves is extremely high and seems to be related to different defense syndromes.

4.2. Summary of defensive syndromes

Given the high vulnerability of young leaves to herbivory, one might expect selection to favor extensive investment in defensive traits. Although there are numerous physical, chemical and developmental options, our data suggest that a given species employs only a subset. Based on data in this paper and from a survey of several Old World forests (Coley and Kursar, 1996), we suggest that the linkages between defenses described above arise because of physiological constraints, such that not all combinations of traits are possible. These tradeoffs have apparently led to the independent evolution of similar defense syndromes in many lineages. Because of tradeoffs between defenses, species fall along the continuum from 'escape' to 'defense' extremes. In Table 2, we summarize the traits associated with these two strategies.

One key tradeoff is between nitrogen content and leaf expansion (Kursar and Coley, 1991). Because young leaves are vulnerable to herbivores until they finish expanding and begin to toughen, rapid expansion should shorten this window and *reduce* herbivory (Aide and Londoño, 1989). However, species with more rapid leaf expansion suffer significantly *higher* herbivory (Table 2, Fig. 2). This counter-intuitive result is because other traits are correlated with rapid expansion. For example, nitrogen is positively correlated with expansion rate (Table 2, Fig. 5), presumably because of increased metabolic demands within the rapidly growing leaf. Species with the most rapid expansion have leaves that double in size in less than a day, an impressive growth rate that presumably requires high levels of enzymes. In contrast, species with slow-expanding leaves have lower nitrogen. Although the ideal defense combination would be fast expansion and low nitrogen, this is physiologically impossible. Thus, the higher nitrogen content of fast-expanding species is unavoidable and likely contributes to their higher rates of herbivory.

Another possible reason why species with rapid expansion of young leaves suffer higher herbivory is because they are less well defended chemically (Table 2). Our data testing the effects of extracts from 24 species, suggests that species with fast

Table 2
Young leaf characteristics for species with 'escape' and 'defense' syndromes

	Escape	Defense
Herbivory	high	low
Toughness	low	low
Leaf expansion rate	fast	slow
Nitrogen for growth	high	low
Chemical defenses	low	high
Chloroplast development	delayed	normal
Nitrogen for greening	low	high
Synchrony of leaf production	high	low

expansion have less effective secondary metabolites (Fig. 3). In feeding trials with herbivores, both caterpillars and beetles showed a strong preference for extracts from species with fast over slow expansion. Lepidoptera and Coleoptera are probably the most important herbivores selecting for plant defenses in tropical rainforests (Janzen, 1988; Barone, 1998). *Schistocerca* showed no significant preference which is consistent with the generalized diet choice associated with most Orthoptera (Marquis and Braker, 1994). Generalist herbivores give an estimate of toxicity and deterrence, and are the only practical approach when comparing many unrelated species. However, bioassays with specialist herbivores could be accomplished within a genus and would provide valuable additional information. Although we have not done this, we predict that on average, specialists would also prefer 'escape' species. All fungal pathogens, including both the leaf-spotting *Pestalotiopsis* as well as the two opportunistic pathogens, grew better on extracts from species with fast leaf expansion. Extracts from species with slow expansion thus appear to be more toxic to a broad range of leaf-attacking organisms.

Although no systematic characterizations of secondary metabolites have been carried out for young leaves with different expansion rates, other evidence supports the hypothesis that species with slow expansion are better defended chemically. In a partial regression with the nutritional effects removed (nitrogen, toughness and water), we still found a significant positive relationship between herbivory and leaf expansion rate for species from BCI, Africa and Southeast Asia (Coley and Kursar, 1996). This is most likely explained by more effective secondary metabolites in species with slow leaf expansion. We found no correlation between leaf expansion rate and phenolic content, nor any obvious patterns with alkaloids or saponins. It seems likely that the greater inhibitory effects of extracts from species with slow expansion may have a complex chemical basis and may be attributed to a number of classes of secondary metabolites.

The advantages of effective chemical defense are obvious, so why would species with fast expansion invest less in secondary metabolites? We suggest that this may be because of competition for resources. For species whose leaves double in size every day, it may simply not be possible to simultaneously allocate resources to rapid growth and chemical defense. Nonetheless, herbivory clearly decreases fitness, so we interpret the strategy of rapid expansion, with its associated high damage rates, as being a sub-optimal strategy. Below we present an evolutionary scenario for how this strategy might have arisen (Section 4.4).

Species with rapid leaf expansion suffer higher rates of herbivory, apparently because of higher nutritional value and lower secondary metabolites. However, one mechanism for reducing the impact of this damage is to delay greening (Table 2). Chloroplasts contain high concentrations of proteins and lipid-rich membranes, greatly adding to the energy and nitrogen content of a leaf. By delaying chloroplast development, a young 'white' leaf invests 15% less energy per dw relative to a young 'green' leaf (Kursar and Coley, 1992a,b). For a given expansion rate, 'white' leaves also contain significantly less nitrogen than 'green' leaves (Figs. 1 and 5). This reduction in resources is not sufficient to discourage herbivores, however, the benefit of delayed greening is that *when* white leaves are eaten, they lose less energy

and nitrogen than they would if they were green. The cost of delayed greening is that leaves maintain low rates of photosynthesis during expansion. In the extremely low light levels of the understory, this is not a large cost and is balanced by reduced resource loss for species with high herbivory. This model correctly predicts that delayed greening should be more common in shade species with rapid expansion as they suffer the highest losses to herbivores (Figs. 1 and 4). Additionally, it may not be possible for leaves to expand quickly and allocate sufficient resources to build expensive photosynthetic machinery.

Synchronous production of young leaves appears to be a mechanism for satiating specialist herbivores and thus lowering rates of herbivory (Aide, 1993). The disadvantages of synchronous flushing are storage costs and opportunity costs for not producing leaves as frequently as possible. Nonetheless, species differ considerably in the synchrony of leaf flushing and there is a positive correlation between expansion rate and synchrony ($r^2 = 0.36$, $P < 0.001$ in Coley and Kursar, 1996). Leaf production is more synchronous in fast-expanding species (Table 2) which are the most vulnerable to herbivores and could therefore benefit from the added protection of synchrony. Slow-expanders suffer little herbivory, so synchrony would not be advantageous.

4.3. Convergent evolution of defense syndromes

We have found the same ‘escape’ and ‘defense’ syndromes across many unrelated species in Africa, Southeast Asia, and the Neotropics, a pattern strongly suggestive of convergent evolution (Coley and Kursar, 1996). This repeated pattern makes sense if only specific combinations of traits are possible or adaptive. We argue that the linkages between defenses described above arise because of physiological constraints and fitness tradeoffs such that only certain combinations of traits are compatible (Table 2). For example, it would be advantageous to have leaves with fast expansion (which reduces the period of vulnerability to herbivores), low nitrogen (which reduces their palatability) and effective secondary metabolites. However, rapid leaf expansion requires high levels of nitrogen and other resources which appear to be reallocated from secondary metabolites and chloroplast development. Thus the optimal combination of traits is not possible. Because traits are linked, we must view each defense in the context of the other co-occurring traits in order to understand the selection pressures that have favored that particular defense, or the consequences of that defense for herbivores.

4.4. Evolutionary scenario

Are the ‘escape’ and ‘defense’ syndromes two equally viable solutions for protecting young leaves? Typically, when we think of tradeoffs, we assume that all combinations of traits along the tradeoff curve have equivalent fitness. In other words, several ways of combining traits can result in equally adaptive solutions. However, in the case of young leaves, ‘escape’ species suffer high losses to herbivory, even taking into account the lower investment in chloroplasts. Thus, we suggest that

'escape' and 'defense' syndromes are not equally fit approaches for protecting young leaves. Why then would selection favor the 'escape' strategy when it appears less effective?

We propose an evolutionary scenario which suggests that the effectiveness of secondary metabolites against herbivores determines whether a species follows a 'defense' or 'escape' trajectory (Fig. 6). Herbivores are continually evolving adaptations to their host plant's secondary metabolites such as the ability to avoid, detoxify or even sequester the plant's defensive compounds. In response, some plant species may evolve novel and more effective chemical defenses leading to an 'arms race' (Ehrlich and Raven, 1964; Kareiva, 1999; Thompson, 1999; Futuyma, 2000; Rausher, 2001). One possible evolutionary outcome for young leaves (depicted in the top panels of Fig. 6) is an 'arms race' between herbivores and plants wherein continual evolution of plant secondary metabolites maintains effective defense against herbivores. Over time, we might therefore expect the evolution of an increasing number of secondary metabolites, greater toxicity, and perhaps more biosynthetically derived compounds if plants are elaborating on existing chemical structures (e.g. Berenbaum, 1978; Berenbaum et al., 1986; Laue et al., 2000). The alternative 'escape' syndrome would evolve only if the secondary metabolites were unable to effectively discourage herbivores. In this case, selection would favor shortening the period of high herbivory by increasing expansion rate. To do this, resources invested in the relatively ineffective secondary metabolites could be shifted to increase expansion rates without any great loss of protection. This could lead to a cycle of increasing expansion rates at the expense of defense. Thus, the escape syndrome seems to be a way that species can minimize damage given the failure of their secondary metabolites to provide protection. Once species are forced down this evolutionary path, it may be difficult to re-enter the chemical arms race. Although our results are consistent with this evolutionary scenario, additional data on chemical defenses, collected within a phylogenetic context, are needed to more fully explore this hypothesis.

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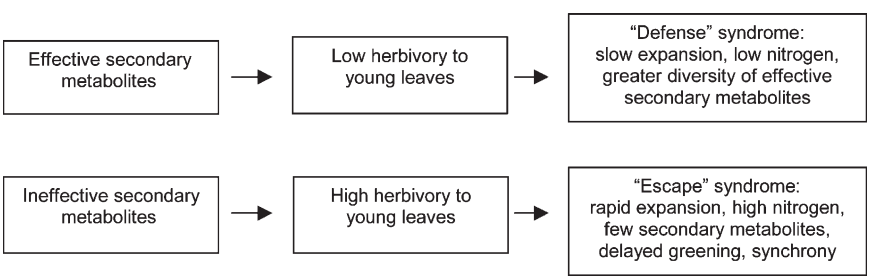

Fig. 6. Evolutionary scenario for young leaves with 'defense' and 'escape' syndromes.

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Appendix 1

Species used (a) to correlate leaf traits with herbivory, (b) to measure daily rates of herbivory and (c) to conduct feeding trials. *Inga* nomenclature follows Pennington (1997), others follow the Missouri Botanical Garden VAST database (Solomon, 2002).

(a) *Inga* species (Fabaceae: Mimosoideae) used for herbivory (h) and chlorophyll (c) analysis.
I. acuminata (Benth.) (h, c)
I. cocleensis (Pitt.) (c)
I. goldmanii (Pittier) (h,c)
I. laurina [(Sw.) Willd.] (c)
I. marginata (Willd.) (h,c)
I. nobilis (Willd.) (h,c)
I. pezizifera (Benth. in Hook) (h,c)
I. sapindoides (Willd.) (c)
I. umbellifera [(Vahl) Steud.] (h,c)
I. vera (Willd.) (c)

(b) Daily rates of herbivory.
Ouratea lucens [(Kunth) Engl.] Ochnaceae, understory tree
Connarus panamensis (Griseb.) Connaraceae, liana
Xylopia macrantha (Triana & Planch.) Annonaceae, understory tree
Desmopsis panamensis [(B.L. Rob.) Staff.] Annonaceae, understory tree
Annona spraguei (Staff.) Annonaceae, light-demanding tree

(c) Feeding trials for species with fast (f) and slow (s) expanding young leaves.
Pair 1 *Paullinia bracteosa* (Radlk.) Sapindaceae (f) *Paullinia rugosa* (Benth. ex Radlk.) Sapindaceae (s)
Pair 2 *Cupania sylvatica* (Casar.) Sapindaceae (f) *Cupania rufescens* (Triana & Planch.) Sapindaceae (s)
Pair 3 *Inga marginata* (Willd.) Fabaceae: Mimosoideae (f) *Inga vera* (Willd.) Fabaceae: Mimosoideae (s)
Pair 4 *Faramea occidentalis* [(L.) A. Rich.] Rubiaceae (f) *Psychotria marginata* (Sw.) Rubiaceae (s)

Pair 5 *Licania platypus* [(Hemsl.) Fritsch] Chrysobalanaceae (f) *Hirtella triandra* (Sw.) Chrysobalanaceae (s)
Pair 6 *Eugenia oerstediana* (O. Berg) Myrtaceae (f) *Myrcia fosteri* (Croat) Myrtaceae (s)
Pair 7 *Ouratea lucens* [(Kunth) Engl.] Ochnaceae (f) *Laetia thamnia* (L.) Flacourtiaceae (s)
Pair 8 *Talisia princeps* (Oliv.) Sapindaceae (f) *Heisteria concinna* (Standl.) Olacaceae (s)
Pair 9 *Garcinia intermedia* [(Pittier) Hammel] Clusiaceae (f) *Piper cordulatum* (C. DC.) Piperaceae (s)
Pair 10 *Prioria copaifera* (Griseb.) Fabaceae: Caesalpinioideae (f) *Psychotria limonensis* (K. Krause) Rubiaceae (s)
Pair 11 *Gustavia superba* [(Kunth) O. Berg] Lecythidaceae (f) *Guatteria dumetorum* (R. E. Fr.) Annonaceae (s)
Pair 12 *Connarus turczaninowii* (Triana & Planch.) Connaraceae (f) *Chrysophyllum panamense* (Pittier) Sapotaceae (s)

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