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THE GROWTH–DEFENSE TRADE-OFF AND HABITAT SPECIALIZATION BY PLANTS IN AMAZONIAN FORESTS

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Abstract. Tropical forests include a diversity of habitats, which has led to specialization in plants. Near Iquitos, in the Peruvian Amazon, nutrient-rich clay forests surround nutrient-poor white-sand forests, each harboring a unique composition of habitat specialist trees. We tested the hypothesis that the combination of impoverished soils and herbivory creates strong natural selection for plant defenses in white-sand forest, while rapid growth is favored in clay forests. Recently, we reported evidence from a reciprocal-transplant experiment that manipulated the presence of herbivores and involved 20 species from six genera, including phylogenetically independent pairs of closely related white-sand and clay specialists. When protected from herbivores, clay specialists exhibited faster growth rates than white-sand specialists in both habitats. But, when unprotected, white-sand specialists outperformed clay specialists in white-sand habitat, and clay specialists outperformed white-sand specialists in clay habitat.

Here we test further the hypothesis that the growth–defense trade-off contributes to habitat specialization by comparing patterns of growth, herbivory, and defensive traits in these same six genera of white-sand and clay specialists. While the probability of herbivore attack did not differ between the two habitats, an artificial defoliation experiment showed that the impact of herbivory on plant mortality was significantly greater in white-sand forests. We quantified the amount of terpenes, phenolics, leaf toughness, and available foliar protein for the plants in the experiment. Different genera invested in different defensive strategies, and we found strong evidence for phylogenetic constraint in defense type. Overall, however, we found significantly higher total defense investment for white-sand specialists, relative to their clay specialist congeners. Furthermore, herbivore resistance consistently exhibited a significant trade-off against growth rate in each of the six phylogenetically independent species-pairs.

These results confirm theoretical predictions that a trade-off exists between growth rate and defense investment, causing white-sand and clay specialists to evolve divergent strategies. We propose that the growth–defense trade-off is universal and provides an important mechanism by which herbivores govern plant distribution patterns across resource gradients.

Key words: Amazon; ecological gradient; growth–defense trade-off; habitat specialization; herbivory; phenolics; phylogenetic control; rainforest; reciprocal-transplant experiment; terpenes; tropical trees.

Introduction

The regional diversity of plant species arises, in part, because a given species is restricted to a subset of environmental conditions. But how and why does this habitat specialization occur? The most common explanation is that habitat specialists are physiologically adapted to growing in their particular abiotic environment and out-compete other plants that are not so closely suited to the local conditions (Ashton 1969, Cody 1978, Bunce et al. 1979). However, herbivore–plant interactions can also contribute to the evolution of habitat specialization. Theoretical work has demonstrated that herbivores can alter competitive relationships among plants, especially when there is spatial heterogeneity of resources (Louda et al. 1990, Grover and Holt 1998). Empirical studies at the population and community levels have documented that herbivores can reduce plants' potential distributions, restricting them to a subset of the habitats that they might physiologically tolerate (Parker and Root 1981, Louda 1982, 1983, Louda and Rodman 1996, Olff and Ritchie 1998,

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Carson and Root 2000, Harley 2003). Thus, herbivores can play a major role in determining which species of plants dominate in a community, as well as in which habitats a species will be successful.

The lowland Amazonian rainforest near Iquitos, Peru provides an ideal system to study habitat specialization and the role of herbivores. Forests in the Iquitos area grow on a mosaic of soil types; including red clay soils and extremely infertile white-sand soils (Kauffmann et al. 1998). The two soil types lie immediately adjacent to each other, the boundaries are well defined, and each soil type is associated with a distinctive flora (Gentry 1986, Vásquez 1997, Fine 2004). White-sand forests are much more resource limited than clay soil forests (Medina and Cuevas 1989, Coomes and Grubb 1998, Moran et al. 2000). Resource availability theory proposes that resource-limited species will have slower growth rates and higher optimal levels of defense, reflecting the decreased ability of a resource-limited plant to compensate for tissues lost due to herbivory (Janzen 1974, Coley et al. 1985, Coley 1987*b*). Thus we predict that species growing in white-sand forests should evolve to allocate relatively more resources to defense than species growing in clay forests (Fine et al. 2004).

Recently, we reported the results of a reciprocal-transplant experiment of 20 species of seedlings from six genera of phylogenetically independent pairs of white-sand and clay specialist plants (Fine et al. 2004). We manipulated the presence of herbivores and found that clay specialists grew significantly faster than did white-sand specialists in both habitats when protected from herbivores. But when herbivores were not excluded, white-sand specialists out-performed clay specialists in white-sand forests, and clay specialists grew faster than white-sand specialists in clay forests. These results strongly supported the existence of a growth–defense trade-off, with habitat specialization being enforced by herbivores (Fine et al. 2004).

Here, we test further the predictions of the growth–defense trade-off by comparing species-level patterns of growth, herbivory, and defense in this same phylogenetically diverse group of tree species. We predicted that closely related species specialized to contrasting soil types should diverge in traits that confer defense vs. those that confer growth. We investigated the evidence for such differential investment while controlling for phylogeny. Therefore, any differences in defense allocation found between closely related white-sand and clay specialists can be inferred to be traits derived for habitat specialization. This phylogenetically controlled approach enabled us to investigate the degree of constraint involved in the type and amount of defense, and to separate this from the repeated and independent evolution of defensive traits due to selection from similar ecological conditions. Second, examining defense investment with a reciprocal-transplant experiment allowed us to identify which traits (if any) are phenotypically plastic as opposed to genetically controlled adaptations to a particular habitat.

Thus, to test whether the growth–defense trade-off contributes to habitat specialization in white-sand and clay forests, we combined field observations and a reciprocal-transplant experiment to ask the following questions: (1) Are there differences in herbivore abundance in the two habitats? (2) Is there a difference in the impact of herbivory in the two habitats, suggesting selection for greater defense investment in white-sand habitats? (3) Do white-sand and clay specialists differ in their type of defensive strategy or in their amount of defense investment? Are these differences phylogenetically constrained or repeatedly and independently evolved? (4) Are defensive traits in white-sand and clay specialists affected by resource-driven phenotypic plasticity? (5) Do white-sand and clay specialists follow the predictions of the growth–defense trade-off?

Materials and Methods

Study site and study species

We conducted this research in the Allpahuayo-Mishana National Reserve near Iquitos, Peru (3°57′ S, 73°24′ W). This 57 600-ha reserve is at ~130 m elevation and receives more than 3000 mm of precipitation during the year, with no distinct dry season (Marengo 1998). Many white-sand specialist trees belong to the same genera as neighboring clay forest specialists, allowing for a phylogenetically controlled experiment using edaphic specialist species. For a reciprocal-transplant experiment, we chose 20 common white-sand and clay specialists from six genera from five families (see Fine et al. [2004] for a phylogeny). The genera were *Mabea* (Euphorbiaceae), *Oxandra* (Annonaceae), *Pachira* (Malvaceae sensu lato), *Parkia* (Fabaceae), *Protium* (Burseraceae), and *Swartzia* (Fabaceae). Each genus was represented by one white-sand specialist and one clay specialist, except for *Protium*, which was represented by six clay specialists and four white-sand specialists. Designation of habitat for each species was accomplished by extensive inventories (Fine 2004, Fine et al. 2005) as well as consultation of local floras and other published species lists from the western Amazon (Vásquez 1997, Ruokolainen and Tuomisto 1998, Jørgensen and Léon-Yánez 1999, García et al. 2003).

Nitrogen availability

To test for differences in nitrogen availability between white-sand and clay habitats, we filled 27 nylon stocking bags filled with 8 g of Rexyn 300 (H-OH) analytical grade resin beads. In May 2002, we placed the ion-exchange resin bags beneath the litter layer and root mat at the organic material–mineral soil interface at our white-sand and clay sites (Binkley and Matson 1983). The bags were collected after five weeks, extracted with KCl, and measured by standard techniques with an autoanalyzer (University of Wisconsin Soils Laboratory). Nitrate, ammonium, and root mat depth differences were tested for significance between soil types with a Wilcoxon signed-ranks test.

The reciprocal-transplant experiment

We used a reciprocal-transplant experiment to test whether white-sand and clay specialists had different growth rates and defense investments as predicted by the growth–defense trade-off hypothesis. In addition, the reciprocal-transplant experiment allowed us to test for phenotypic plasticity of defense investments under different edaphic and herbivore treatments.

In May 2001, we built 22 control and 22 herbivore exclosures (3 × 3 × 2 m); half were located in clay forest and half in white-sand forest. We transplanted 880 seedlings from the six genera into the controls and exclosures (see Fine et al. 2004). Using the results of the reciprocal-transplant experiment reported in Fine et al. (2004), we compared the amount of leaf and height growth of the plants grown in herbivore exclosures to the unprotected controls, and estimated the effect herbivory had on growth rates for each white-sand and clay specialist. This measure is referred to throughout as "protection effect." The experiment lasted until February 2003 (21 mo after transplanting, 18 mo after first data collection), at which point leaves were collected to measure defensive traits.

Insect abundance and species richness

To evaluate differences in insect abundance and composition across habitats, we used a portable black light attached to a battery to attract insects in five white-sand and five clay sites. During 8–20 December 2002, on rain-free evenings between 1900–2000, the black-light was illuminated and suspended above white sheets. We collected all insects from the families/orders Blattoideae, Coleoptera, Hemiptera, Homoptera, and Orthoptera. We excluded all obvious predators and collected all herbivorous insects from these five groups and counted and identified them to order and family and then separated them into morphospecies. Parasitoid wasps were collected with malaise traps over a two-year period in 15 white-sand and nonwhite-sand forest sites in the Allpahuayo-Mishana National Reserve (from 15 of the same sites described in Fine [2004]) as a part of a much larger study on ichneumonid wasps (for detailed methods see Sääksjärvi [2003]). Since these parasitoid wasps attack either herbivorous insects (or predators of herbivorous insects), we would expect parasitoid diversity and abundance to track herbivorous insect diversity and abundance in white-sand and clay forests. To test for differences between white-sand and clay habitats (both the black light trap data and the wasp data), Wilcoxon signed-ranks tests were conducted on the ranked abundances and numbers of species.

Field herbivory

For herbivory comparisons in addition to those from the transplant experiment, we chose six genera that were common in both white sand and clay forests: *Protium* (Burseraceae), *Hevea* and *Mabea* (Euphorbiaceae), *Pachira* (Malvaceae s.l.), and *Macrolobium* (Fabaceae). In September 2000, in the same white-sand and clay sites where the wasps were collected, we sampled 355 individuals in the field from >20 species of *Protium*, two species of *Hevea*, two species of *Mabea*, two species of *Pachira*, and three species of *Macrolobium*. Most of these species were found in only one of the two habitats. Plants were 1–3 m tall (juvenile trees). We marked newly expanding leaves (or leaves that had already expanded but were not toughened) with small colored wires, from 1–10 leaves or leaflets per plant. After five to seven weeks we estimated the amount of leaf area missing from the marked leaflet (0–100%). Average amount of leaf area missing was divided by number of days between marking and the census (damage per day). These data were arcsine square-root transformed to improve normality, and a mixed-model ANOVA (including the random factor genus and the fixed factor habitat) was performed on the data to test for differences in herbivory rate between white-sand and clay habitats.

Impact of herbivory (defoliation experiment)

In February 2003, after collecting leaf material for chemical analyses from all of the seedlings in the reciprocal-transplant experiment, we removed 100% of the remaining leaves to test the effect of defoliation on white-sand and clay specialists in the two habitats. After three months, we counted the number of seedlings that survived defoliation. To compare mortality rates, we averaged mortality for white-sand specialists and clay specialists in each of the 44 controls and exclosures (*Protium* species in each control and exclosure were weighted to give each genus equal importance in the analyses). A fixed-factor ANOVA including the terms habitat (white-sand or clay), origin (white-sand or clay specialist), and the origin × habitat interaction was used to assess the effects of origin and habitat on mortality due to defoliation. Post hoc tests on the individual group means were performed using the studentized t distribution (appropriate for equal sample sizes; Sokal and Rohlf 1995).

Defensive characteristics of white-sand and clay specialists

Comparing differences in herbivory and growth is the best method of comparing defense investment in white-sand and clay specialists, since this approach takes into account the entire arsenal of plant defenses as experienced by the actual herbivores (cf. Simms and Rausher 1987). However, to investigate which particular defensive traits are deterring herbivores, we measured two classes of chemical defenses, a physical defense, and the nutritional quality of white-sand and clay specialists. After the transplant experiment was completed, we collected leaves from all surviving plants to compare defense investment in white-sand and clay specialists, and to assess the effect of habitat and treatment on the plasticity of defense investment for each species. We collected marked mature leaves that were produced after plants were transplanted. We measured terpenes, total phenolics, toughness, and available protein for all seedlings in the reciprocal-transplant experiment. Terpenes and phenolics are carbon-based secondary compounds common to many families of plants, including those in our research (Mabry and Gill 1979, Bernays et al. 1989, Schultes and Raffauf 1990). Although phenolics and terpenes have alternative functions, they commonly function in herbivore deterrence (Mabry and Gill 1979, Bernays et al. 1989, Herms and Mattson 1992, Langenheim 1994; but see Harborne 1991, Close and McArthur 2002). Increased toughness of leaves (sclerophylly) is a mechanical antiherbivore defense that is commonly found worldwide in plants that live in resource-limited environments (Coley 1983, 1987*a*, Grubb 1986, Turner 1994). Finally, available foliar protein is a good measure of a plant's nutritional quality. Moran and Hamilton (1980) hypothesized that plant nutrition can be considered a defensive trait if it can be selected for by herbivore attack. This can result if herbivores detect nutritional differences and prefer plants with higher nutrition (cf. Scheirs et al. 2003). A second mechanism is if slow growth by herbivores due to low nutrition results in higher predation rates (cf. Denno et al. 2002).

Chemical defenses

To compare terpene investment among the species, ~500 mg (fresh mass) leaves from the experimental seedlings were collected at the experimental sites in 2-mL glass vials and filled with dichloromethane (DCM). This leaf–DCM mixture was used for qualitative and quantitative analyses with gas chromatography (GC) and gas chromatography–mass spectrometry (GCMS). (See Appendix A for detailed methods of terpene extraction and analysis.)

For comparisons of total phenolics, ~2 g fresh mass of mature leaves of 16 individuals (8 protected and 8 unprotected) from each species in the reciprocal-transplant experiment were collected and immediately placed in plastic tubes containing silica gel desiccant. Leaves were later analyzed for phenolic compounds in the Appel/Schultz laboratory at Penn State University. Whenever possible, bulk tannins were prepared to provide standards for the phenolic assays of individual samples. This is a crude purification, and although nonphenolic materials are unlikely to be present (Hagerman and Klucher 1986; H. M. Appel and J. C. Schultz, *unpublished data*), the product is merely a more representative sample of extractable polyphenols found in the actual plant than is a commercial standard from some other source. (See Appendix A for detailed information on all methodology of phenolic extraction, purification, and analysis.) Because total phenolics likely function as an antiherbivore defense by precipitating available protein (Herms and Mattson 1992), we divided our total phenolics obtained as described with available foliar protein data to create a phenolic : protein ratio (Nichols-Orians 1991).

Leaf toughness

A "penetrometer" (Chatillon Universal Tension and Compression Tester, Largo, Florida, USA) was used to puncture holes through the leaf (or leaflet) lamina to give a measure of toughness. It was impossible to test the pair of species from the genus *Parkia*, since both have bipinnately compound leaves, with leaflets not much larger than the 3 mm diameter of the testing machine's rod. We standardized the punch position to midway between leaf tip and base, between the midrib and the leaf margin, avoiding the main veins where possible. The punch test measures a combination of shear and compressive strength and resistance to crack propagation. For these reasons, it has been criticized as not specifically measuring leaf toughness (Choong et al. 1992). Nevertheless, it is easy to perform in the field and highly correlated with more specific tests to measure the physical properties of leaf toughness (shearing and tearing parameters) (Edwards et al. 2000).

Soluble protein assays

The amount of available foliar protein was measured at the Appel/Schultz laboratory using the same dried-leaf samples collected for the phenolics analyses. (See Appendix A for detailed methods.)

Statistical analyses of growth and defensive traits

Clay and white-sand specialists in each of the six genera were the experimental unit. Because there were four white-sand specialists and six clay specialists from the genus *Protium*, the responses for all *Protium* individuals were weighted to give each genus equal importance in the analysis. The four white-sand specialist *Protium* species were weighted at 0.25, the six clay specialist *Protium* species were weighted at 0.167, and species from all other genera were weighted at 1. We used fixed factor ANOVAs to test for genus, origin (the difference between white-sand specialists and clay specialists), habitat (whether species responded differently depending on where they were planted), and treatment (whether defense investment differed depending on whether the plants were exposed to herbivores). Since we had a priori knowledge that different genera would have different defensive strategies (i.e., some species have terpene investment, others do not), we used fixed-factor ANOVAs for defensive traits (genus was treated as a fixed factor), since our ability to generalize our results in these analyses to unsampled genera is limited. Subsequent to the overall test, individual group means were compared with Tukey hsd post hoc tests.

Defense index

Because different species of plants are likely to employ different defensive strategies, we therefore devised a simple method to combine all measures of chemical defense, leaf toughness, and available protein to investigate whether, for each genus, white-sand specialists were more defended than clay specialists. Values for phenolics, terpenes, and leaf toughness were averaged across both habitats and Z-transformed to give the defense traits among the six pairs of white-sand and clay specialists a mean of zero and a standard deviation of one. Missing data was scored as zero. For available protein, we standardized the inverse of the species averages, because a larger amount of available protein corresponds to lower defense. All four standardized defense variables were then summed to create a defense index (DI). For each genus, the DI for the clay specialist was subtracted from the DI of the white-sand specialist. This method has the assumption that each of these four measures has equal weight, which is undoubtedly incorrect, but preferable than subjectively assigning different weights to defense types. These difference scores ($\mathrm{DI}_{\mathrm{WS}} - \mathrm{DI}_{\mathrm{CL}}$) were used to test the prediction that white-sand specialists are more defended than clay specialists with a one-tailed Wilcoxon paired signed-ranks test (Zar 1999).

Phylogenetic independence of growth, herbivory, and defense traits

In order to evaluate whether growth, herbivory, and defense traits were more similar in closely related genera, we mapped each of the indices listed above, as well as each individual defensive trait onto a phylogeny representing the relationships among the six genera and 20 species (see Fine et al. 2004). Using the program Phylogenetic Independence 2.0, we tested whether traits exhibited significant phylogenetic independence by comparing the average contrast values (C-stat) among the actual trait values for the plant species to the distribution of contrast values created by randomly placing the trait values at the tips within the topology 2000 times and testing for serial independence (TFSI) (Abouheif 1999). If a trait is significantly phylogenetically constrained, then the average C-stat for the actual value will be greater than 95% of the average contrast values generated by the randomization.

Correlations of growth, defense, and herbivory data for the six genera

Species averages for growth (leaf area and height, averaged across habitats), the effect of herbivore protection on growth (arithmetic difference between the average leaf area and height with and without protection, for each white-sand and clay genus averaged across habitats) and defenses, as described, were Z-transformed and analyzed by a method analogous to phylogenetically independent contrasts (Harvey and Pagel 1991). To test for trade-offs, we plotted the values for the six species pairs and analyzed the six slopes, to see if the relationship between traits was consistent when controlling for phylogenetic relationship. We used these plots to test our predictions that (1) growth and herbivory would be positively correlated, (2) growth and defense would be negatively correlated, and (3) herbivory and defense investment would be negatively correlated. Hypotheses about the correlations of traits were tested by the difference scores of the slopes and were evaluated for significance with one-tailed Wilcoxon paired sign-rank tests (Zar 1999).

RESULTS

Differences in nutrient availabilities

Clay forest sites contained significantly more available nitrogen ($Z = 3.53$, $P < 0.0004$) than white-sand forests, more than twice as much available ammonium ($Z = 2.71$, $P < 0.0061$), more than an order of magnitude more available nitrate ($Z = 3.59$, $P < 0.0003$), and a much thinner root mat ($Z = 4.89$, $P < 0.0001$; Table 1).

Habitat differences in herbivore abundance

We found no significant differences in herbivore abundance or species richness between habitats for all herbivores or any of the six orders of herbivorous insects that we collected ($P > 0.05$, Wilcoxon signed-ranks tests; Table 1). Of the 311 morphospecies collected, 208 were collected only once (67%). Of the morphospecies collected more than once, 41 were collected only in white-sand forest, 28 were collected only in clay forest, and 34 were collected in both forests (33% of the morphospecies collected more than once). For parasitoid wasps, no statistical differences in abundance or morphospecies diversity were found between white-sand and nonwhite-sand forest sites (Table 1). Moreover, in the reciprocal-transplant experiment, mean effect of protection for white-sand and clay specialists did not change between habitats (Fig. 1a, b).

Differences in the magnitude of herbivore attack

Clay specialists showed an average increase in growth of 0.25 cm^2/d in leaf area (paired t test, $\mathrm{df} = 5$, $t = -2.91$, $P < 0.05$) and 0.0018 cm/d in height (paired t test, $\mathrm{df} = 5$, $t = -2.59$, $P < 0.05$) when protected from herbivores, while white-sand specialists grew just as well or better in the unprotected vs. protected treatments. When the effect of herbivore protection on leaf area and height growth are Z-transformed and summed, all genera show the same pattern that clay specialists received a greater benefit from herbivore protection than did white-sand specialists.

During our study of field herbivory rates in the two habitats, plants in clay forest sites suffered more than twice the herbivory rates on their new leaves than did plants in white-sand sites (mixed model ANOVA, $F_{1,349} = 6.69$, $P < 0.01$). Clay plants lost almost 23% of their new leaves per month, while white-sand plants lost slightly >10% (Table 1).

Habitat differences in the impact of herbivory

As predicted, seedlings overall suffered higher mortality due to total defoliation in white-sand habitat than

TABLE 1. Comparisons of white-sand and clay forests for leaf litter depth, nitrogen availability, young-leaf herbivory, and insect abundance and morphospecies richness (means ± SE reported).

Variable	Clay forest sites	White-sand forest sites
Root mat (cm) ($N = 44$ plots)	0.91 ± 1.0^{a}	8.48 ± 0.6 ^{b}
Nitrogen availability (ppm) from ion-exchange resin bags ($N = 27$ resin bags)		
NO_3^-	349.2 ± 25.7 ^{b}	25.6 ± 13.8 ^{a}
NH_4^+	135.2 ± 32.7 ^{b}	62.1 ± 17.5 ^{a}
Total nitrogen	484.4 ± 43.0 ^{b}	87.7 ± 23.0 ^{a}
Herbivory (% leaf eaten/mo) ($N = 355$ individuals)	22.8 ± 4.3 ^{b}	10.3 ± 3.3 ^{a}
Insect herbivore abundance ((no. individuals)·(light trap)$^{-1}$·h^{-1})		
Total insect herbivore abundance	87.2 ± 12.6 ^{a}	74.8 ± 18.1 ^{a}
Blattoid abundance	3.0 ± 0.7 ^{a}	2.6 ± 0.9 ^{a}
Coleopteran abundance	20.0 ± 9.0 ^{a}	22.4 ± 7.8 ^{a}
Hemipteran abundance	7.6 ± 4.9 ^{a}	13.4 ± 11.7 ^{a}
Homopteran abundance	20.0 ± 4.5 ^{a}	17.0 ± 1.9 ^{a}
Orthopteran abundance	36.6 ± 8.2 ^{a}	19.2 ± 3.6 ^{a}
Insect herbivore species richness ((no. morphospecies)·(light trap)$^{-1}$·h^{-1})		
Total insect herbivore morphospecies	45.0 ± 4.3 ^{a}	34.8 ± 3.9 ^{a}
Blattoid morphospecies	2.6 ± 0.5 ^{a}	2.4 ± 0.8 ^{a}
Coleopteran morphospecies	7.6 ± 2.1 ^{a}	8.0 ± 2.0 ^{a}
Hemipteran morphospecies	3.2 ± 1.0 ^{a}	2.0 ± 0.4 ^{a}
Homopteran morphospecies	15.8 ± 2.8 ^{a}	11.4 ± 2.0 ^{a}
Orthopteran morphospecies	15.8 ± 1.7 ^{a}	10.8 ± 2.1 ^{a}
Parasitoid wasp ((no. individuals)·site^{-1}·(malaise trap)$^{-1}$ for 2 yr)		
Total parasitoid wasp abundance	67.7 ± 28.5 ^{a}	59.9 ± 10.8^{a}
Total parasitoid species and morphospecies	25.5 ± 6.4 ^{a}	22.0 ± 3.3 ^{a}

Note: Significant differences between forests are indicated by different superscript letters within a row (mixed-model ANOVA, effect of habitat for herbivory, Wilcoxon signed-ranks tests between habitats for litter depth, nitrogen availability, insect abundance, and species richness).

they did in clay habitat (effect of habitat, $F_{1,84} = 4.96$, $P < 0.05$). In addition, white-sand specialists suffered significantly more mortality than did clay specialists in both habitats (effect of origin, $F_{1,84} = 22.8$, $P < 0.0001$; Fig. 2).

Differences in defense investment

Type of defense.—We found strong evidence for phylogenetic constraint for type of defense. The main effect of genus was always significant for differences in terpenes, phenolics, leaf toughness, and available foliar protein. Moreover, it is clear that different genera are relying on different defense strategies, as each of the six genera had a distinct defense investment pattern (see Appendix C). For example, only two genera, *Oxandra* and *Protium,* contained measurable terpenes identified by GCMS (Appendix C). Similarly, only two genera, *Pachira* and *Parkia* had white-sand specialists with obviously tougher leaves than clay specialists. The pattern of high phenolic investment and low available foliar protein in white-sand specialists was more consistent across the six genera, but still there were exceptions (*Oxandra* and *Protium* for phenolics, *Mabea* for available protein; Fig. 3).

Whereas different genera invest in different defensive strategies, we found no consistent relationship between any particular defensive traits that would suggest either a negative trade-off or a synergistic relationship between defense types (Fig. 3).

Amount of defense investment.—We found that five-sixths of the genera have a higher defense index (DI) in the white-sand congener than in the clay congener, and that our prediction of higher defense in the white-sand is supported (one-tailed Wilcoxon paired signed-ranks test, $T_{0.05(1),6} = 1$, $P < 0.05$, Fig. 3).

For phenolic compounds, white-sand specialists overall had significantly higher values for both total phenolics (effect of origin, $F_{1,292} = 50.3$, $P < 0.0001$) and phenolic : protein ratios ($F_{1,292} = 128.2$, $P < 0.0001$) with, respectively, three-sixths and four-sixths of the genera exhibiting significant relationships in the predicted direction (Fig. 3, see Appendix D). The two genera that invested in terpenes, *Protium* and *Oxandra*, exhibited very different patterns of terpene investment in their white-sand and clay specialists (see Appendix D). *Oxandra xylopiodies*, the clay specialist, had significantly higher sesquiterpene and total terpene concentrations than *O. euneura*, the white-sand specialist ($P < 0.05$, Tukey tests; see Appendix D). In contrast, *Protium* white-sand specialists had higher monoterpene and total terpene concentrations than did *Protium* clay specialists ($P < 0.05$, Tukey tests; see Appendix D). Both *Oxandra* and *Protium* white-sand species had significantly higher concentrations of diterpenes and other resins than did their respective clay specialists (see Appendix D).

There was no overall effect of origin on leaf toughness (see Appendix D). In contrast, white-sand species had lower available protein in their leaves than clay specialists (significant effect of origin, $F_{1,292} = 393.5$, $P < 0.0001$; see Appendix D).

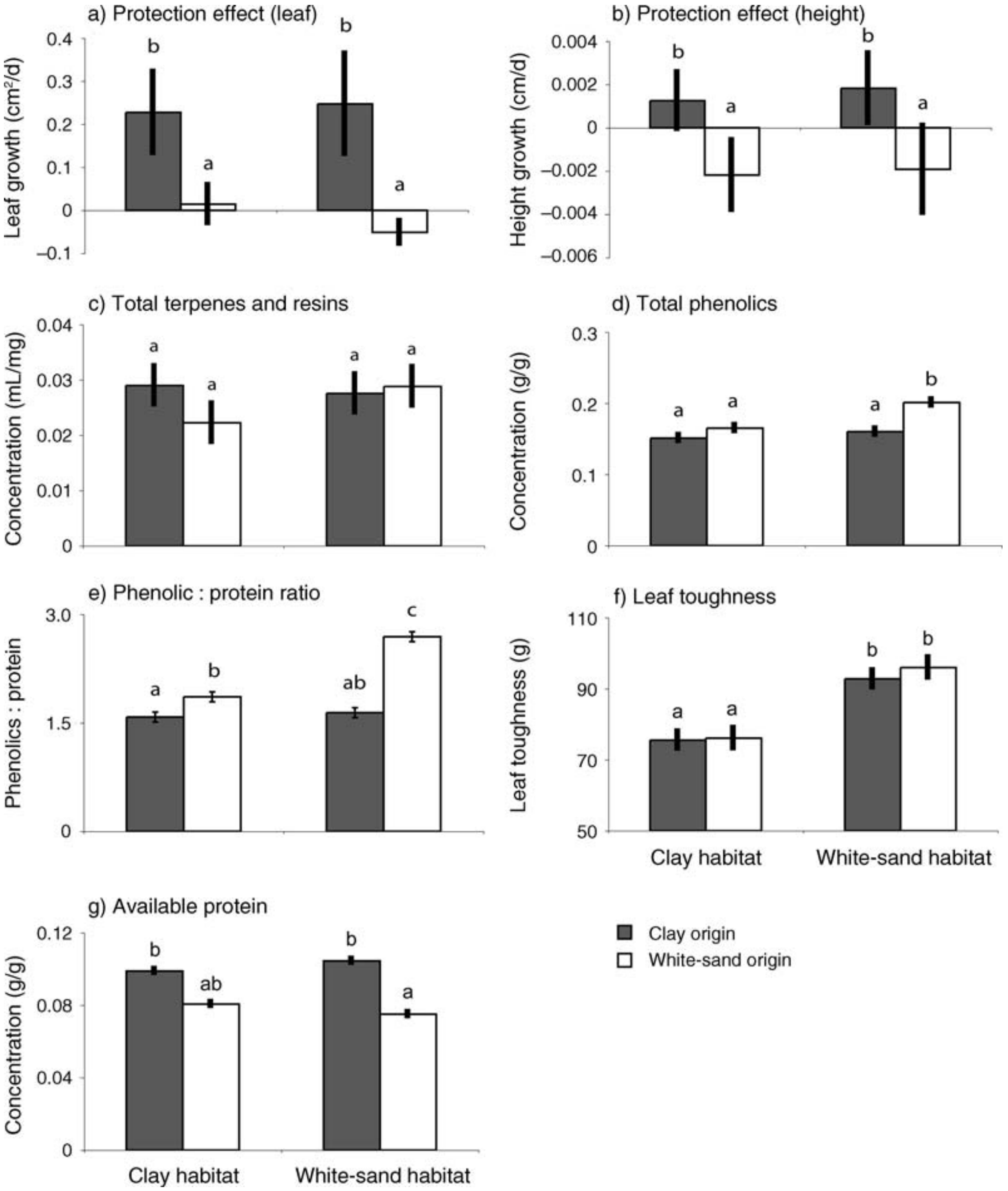


FIG. 1. The effect of origin and habitat in the reciprocal-transplant experiment for (a) the effect of herbivore protection on leaf growth (cm^2/d), (b) the effect of herbivore protection on height growth (cm/d), (c) total terpenes and resins (mL terpenes/mg of dry leaf material), (d) total phenolics (g phenolics/g dry leaf material), (e) phenolic : protein ratio (phenolics divided by available protein, a unitless ratio), (f) leaf toughness (grams of mass to punch a 3-mm rod through a leaf; 1.0 g = 1.38 kPa), and (g) available protein (g soluble protein/g dry leaf material). Histograms show means ± SE. Different letters above bars indicate significant differences among the different groups (Tukey tests).

Defensive traits and phenotypic plasticity

There was no significant overall effect of habitat for terpenes (Fig. 1c). Aside from the outlier behavior by one species, there was no evidence of phenotypic plasticity in phenolic investment (Fig. 1d). *Swartzia cardiosperma* is the only species of 20 in the experiment that showed a significant effect of habitat for phenolic : protein ratios (see Appendix C).

The effect of habitat on leaf toughness was highly significant ($F_{1,388} = 51.6$, $P < 0.0001$; Fig. 1f). Sixteen of 17 species measured had greater leaf toughness in white-sand than clay habitat; three of those were significant (see Appendix C). In contrast, even though nitrogen availabilities differed by more than five times in the two habitats, there was no significant effect of habitat on available protein for either white-sand or clay specialists (Fig. 1g).

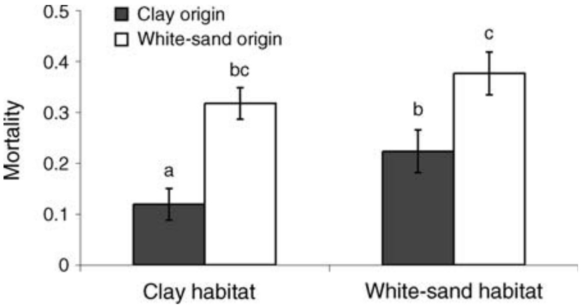


FIG. 2. Mortality results of the 100% defoliation experiment. Bars show average mortality (±SE) for each origin and habitat combination. Different letters above bars indicate significant differences (post hoc tests, studentized *t* distribution).

Evaluating the trade-off: growth vs. defense vs. herbivory

The growth index (GI) and the herbivory index (HI) showed a significant positive relationship (all six of the genera with positive slope, $T_{0.05(1),6} = 0$, $P < 0.025$; Fig. 4a). There was a significant negative trade-off between GI and total DI, with five-sixths of the genera showing a negative slope ($T_{0.05(1),6} = 1$, $P < 0.05$, Wilcoxon paired signed-ranks test; Fig. 4b). Finally, DI showed a significant negative relationship with HI ($T_{0.05(1),6} = 1$, $P < 0.05$; Fig. 4c).

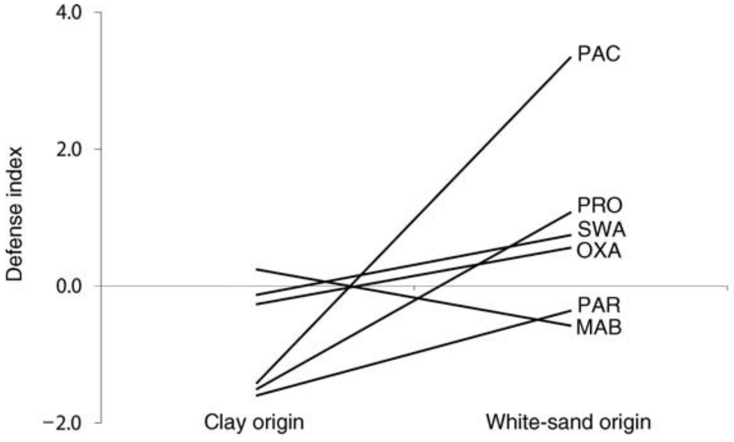
Phylogenetic independence of growth and defensive traits

There was evidence for significant phylogenetic dependence for total phenolics (C-stat = 0.34, $P < 0.002$), terpenes (C-stat = 0.34, $P < 0.002$), and leaf toughness (C-stat = 0.32, $P < 0.012$). The defense index (C-stat = 0.23, $P < 0.11$) and available protein (C-stat = 0.11, $P < 0.148$) exhibited a trend toward phylogenetic constraint. We found no evidence for phylogenetic constraint in GI (C-stat = 0.07, $P < 0.35$) and the protection effect index (C-stat = 0.01, $P < 0.399$), results that in part might reflect an artifact of our design because our sampling within each genus was limited to paired white-sand and clay specialists, which maximized the variation between closely related species.

DISCUSSION

Habitat differences in herbivore populations

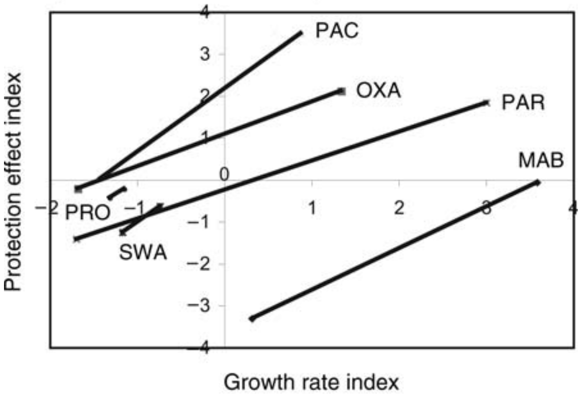
Two separate measures of herbivorous insect communities found statistically similar diversity and abundance in the two forest types. In addition, a full third of the morphospecies that were collected more than once occurred in both habitats. These results are likely explained by the large home range and dispersal capabilities of many herbivorous insects (Stork 1988), coupled with the fact that most white-sand forest



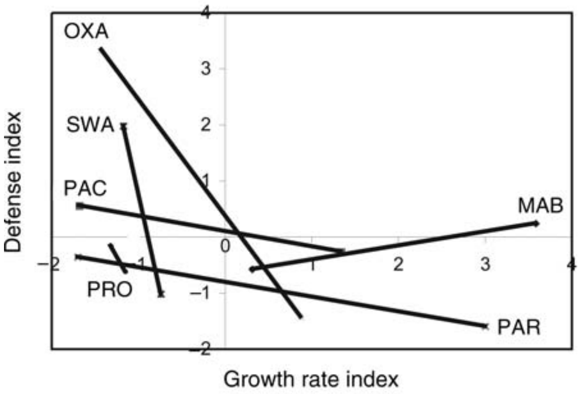
	Terpenes	Tannins	Toughness	Protein	$DI_{ws} - DI_{CL}$
Mabea		■	▨		−0.818
Oxandra	▨	▨		■	0.824
Pachira			■	■	4.749
Parkia		■	■	■	1.234
Protium	■	▨			2.577
Swartzia		■		■	0.870

FIG. 3. The defense index (DI) scores for each genus are plotted, showing the difference between clay (CL) and white-sand (WS) specialists. The three-letter labels of the lines correspond to the genus table below the plot. Black boxes in the table indicate a significantly higher defensive trait for that genus in the white-sand specialist, and shaded boxes indicate a significantly higher defensive trait for the clay specialist (contrary to predictions). The final column shows the DI scores for each genus, with a negative number signifying a score in the direction contrary to predictions.

a) Growth rate vs. herbivory



b) Growth vs. defense



c) Herbivory vs. defense

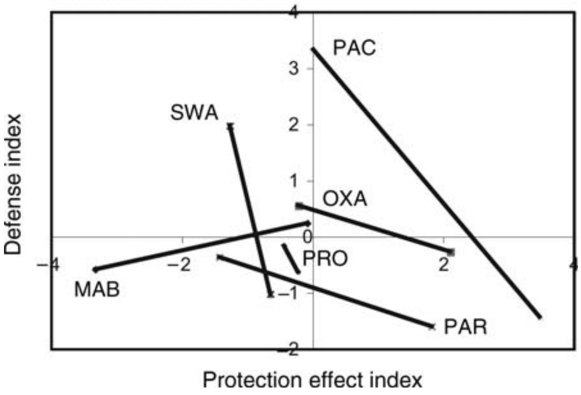


FIG. 4. Plots of the six species pairs for (a) growth rate index vs. protection effect index, (b) growth rate index vs. defense index, and (c) herbivory vs. defense index. The consistency and magnitude of these slopes were used to test the predictions of the growth–defense trade-off hypotheses. The three-letter labels correspond to the six genera listed in Fig. 3.

habitats in the Iquitos area are only a few square kilometers. It is important to recognize that our herbivore sampling was extremely limited and precludes us from drawing definitive conclusions concerning the relative abundance of herbivore populations in white-sand and clay habitats. Nevertheless, our herbivore estimates represent two independent corroborations that insect herbivores indeed range into white-sand forests. Moreover, these patterns are consistent with our herbivory data from the reciprocal-transplant experiment showing that clay specialist seedlings were attacked at similar frequencies whether they were transplanted into clay or white-sand forests (Fig. 1a, b).

Habitat differences in the impact of herbivory

We predicted that the impact of herbivory would be greater in a white-sand forest, because it is more difficult for plants to replace the nitrogen lost to herbivores (Coley 1987*b*, Craine et al. 2003). This prediction was supported by the fact that all plants transplanted into white-sand forest had significantly higher mortality when defoliated than those transplanted into clay forest (Fig. 2).

In the defoliation experiment, white-sand specialists suffered a significantly higher mortality rate than did clay specialists (Fig. 2), confirming a key prediction of the growth–defense trade-off that white-sand specialists ought to have more difficulty replacing tissue lost to herbivores (Coley et al. 1985). This differential response to defoliation by species adapted to low-fertility soils vs. species adapted to high-fertility soils was also found in a study in Singapore (Lim and Turner 1996). Thus, when heavily defended white-sand species are defoliated, they lose costly leaves that represent a high percentage of their energy budget. Due to their slow growth rate, they are then unable to compensate, and this in turn increases their mortality rate (Coley et al. 1987*b*). For this reason the impact of herbivory appears to be substantially greater for plants adapted to low-resource conditions.

Differences in defense investment

Type of defense.—Different genera adopted dramatically different defensive strategies. There was a consistent signal of phylogenetic constraint in our analyses of plant defenses, as genus was a significant factor in each defense variable (see Appendices B and D), and tests for phylogenetic independence confirmed this. In terms of terpenes, phenolics, toughness, and low nutrition, there was no consistent "syndrome" of defensive investment in the six genera; instead, each genus allocated to different combinations of these (and presumably other unmeasured) traits. Indeed, there is little theoretical or empirical support for the idea of a general negative trade-off between types of defensive strategies (Koricheva et al. 2004, Agrawal and Fishbein 2006).

Amount of defensive investment.—For *Protium*, we found higher concentrations of terpenes in white-sand specialists as predicted, but for *Oxandra* the reverse pattern was found (Fig. 3). The terpene profile of *Oxandra* is driven by sesquiterpenes, which could possibly be serving a function other than defense, or do not function in a dosage-dependent fashion (Gershenzon and Croteau 1991, Langenheim 1994). In contrast to sesquiterpenes, both *Protium* and *Oxandra* white-sand specialists were found to have higher diterpenes and other resins compared to clay specialists (see Appendix B). Diterpenes are not volatile and are thought to be either toxic (Lerdau and Penuelas 1993) or a type of physical defense against herbivores or pathogens (Langenheim 1994).

Total phenolics and phenolic : protein ratios were significantly higher overall for white-sand specialists than for clay specialists (see Appendix B). In our study, percentage dry mass of total phenolics ranged 3–37%, a large range that is certainly an overestimate and highlights the difficulty of precise phenolic quantification in the laboratory (Appel et al. 2001). Finally, we found significantly less available protein in white-sand specialists. This was the most consistent trait, with five-sixths of the species pairs showing the same pattern (see Appendix D).

Defensive traits and phenotypic plasticity

We did not find many cases of phenotypic plasticity in the seedlings' allocation to chemical defenses. Very few species had significant increases or decreases in terpenes or phenolics due to habitat (Fig. 1c, d). Similarly, available foliar protein did not change depending on where the seedlings were planted (Fig. 1g), even though nutrient levels were significantly different between the two habitats. We conclude that, for the genera in our study, herbivore resistance due to chemical defenses and available protein content is due to genetically based, fixed traits (but see Boege and Dirzo 2004). Thus, defense differences result from natural selection by herbivores and are not just passive responses to differences of available nutrients in the soils.

In contrast to our results with chemical and nutritional defenses, we found a strong overall effect of habitat on leaf toughness, which was significant for three species (Fig. 1f; see Appendix C). Overall, we found that leaf toughness was significantly higher for white-sand species in only two genera, *Parkia* (which we were not able to measure with our penetrometer, but for which the pattern was obvious) and *Pachira*. In contrast, two previous studies found that white-sand plants had significantly tougher leaves than clay plants (Coley 1987*a*, Choong et al. 1992). These studies did not take phylogeny into account, but their results for white-sand and clay species averages were much more divergent than ours. One possibility for the discrepancy is that toughness in these two studies was only measured in the plants' home habitats. While our results in no way negate the potentially strong selective effect of herbivores on sclerophylly, they do suggest that future comparisons of white-sand and clay species should not only be controlled for phylogenetic relationships, but also for resource availability.

Evaluating the growth–defense trade-off

The evolutionary trade-off between growth and defense is illustrated by the data graphed in Fig. 4. When the protection effect of each species is plotted against the overall growth rate (Fig. 4a), all six genera exhibited a positive relationship. In each genus, herbivores selectively attacked the faster growing species more than the slower growing species. This is evidence that faster growing plants have lower resistance to herbivores, consistent with the predictions of the growth–defense trade-off. Coley (1983, 1987*b*) found the same relationship in Panama where the growth rates of 40 species of trees were positively correlated with their rates of herbivory.

In the graphs of Fig. 4a, the lengths of the lines correspond to the amount of variation in growth rate and antiherbivore traits within the species (white-sand and clay) of each genus. For example, some genera like *Parkia* are represented by longer lines in the horizontal direction (Fig. 4a), because this genus includes both shade-tolerant species and those that thrive in high-light conditions. Therefore, the clay specialist in *Parkia* is a very fast grower relative to the *Protium* and *Swartzia* species, all of which are shade-tolerant species and never found in tree-fall gaps (P. V. A. Fine, *personal observation*). Yet the fact that the slopes of the six lines in Fig. 4a are so similar suggests the existence of a universal trade-off, even among species with such disparate growth rates and defensive strategies.

When the defense index (DI) scores for the six genera are plotted against their growth rate index (GI) (Fig. 4b), we found a significant negative relationship, with five of the six genera having higher DI scores in the slower vs. faster growing species. The slopes in this graph exhibit much more variation than the growth vs. herbivory graph (Fig. 4a), likely due to the coarse method by which we attempted to quantify defense investment in these species. The one outlier genus, *Mabea*, shows the opposite relationship than the other five genera, with a higher DI score in its faster growing, clay specialist. Because the slower growing (white-sand specialist) *Mabea* received the least amount of attack from herbivores in the experiment (see Appendix B), it seems likely that it actually is very well defended and we failed to accurately quantify its defensive investment. One reason for this may be that *Mabea* is the only genus of the six that produces copious white latex, and we did not quantify this trait in our comparisons. The herbivory vs. defense graph (Fig. 4c) echoes this point, with *Mabea* the only genus whose DI score does not match its herbivory index score.

Phylogenetic approach to studying the growth–defense trade-off

Our approach using multiple pairs of congeners from ecologically divergent habitats differs from some other more quantitative approaches that have used data on branch lengths from a phylogenetic tree to test for correlations between particular traits and habitat association (Cavender-Bares et al. 2004). In our approach, we ignore branch lengths by design, since each of our genus pairs includes just one representative from each habitat type. But in terms of comparisons of growth rate, herbivory, and *overall* defense as it relates to white-sand and clay specialization, our results indicate that variation in branch lengths among our pairs matters very little: All six pairs exhibit similar trade-offs (Fig. 4a). Moreover, if this trade-off has a bearing on a plant's distribution onto white-sand and clay soils, then evidence for it must be present both in the most recently derived specialist pairs as well as in pairs of species that have persisted for millennia in their particular habitats. By contrast, if we were interested in the evolution of particular traits (like phenolics per se), then inclusion of some estimate of divergence time (and denser sampling within genera) would certainly be warranted.

One limitation of the congeneric pair approach is that one's sample is limited to genera that include species that occur in both of the habitats of interest. It would be interesting to compare genera that were restricted to only white-sand or only clay habitats to see if the growth–defense trade-off was evident in comparisons with their sister taxa (that were specialists to the contrasting habitat). Our way of calculating a defense index (DI) works well precisely because defensive traits are phylogenetically conserved between close relatives, allowing for quantitative comparisons of the same qualitative trait. If we used pairs of taxa that were not closely related, it would become much more difficult to capture the defense allocation of each contrasting species within a DI, although protection effect would still be an appropriate measure for comparison.

Including a phylogenetic context is vital for studies of the growth–defense trade-off for at least two reasons. First, controlling for phylogeny is critical because it reduces the noise of interspecific variation that can easily obscure the true patterns in the data (Agrawal and Kotanen 2003). For example, there is substantial variation in both growth and herbivory rates among these six species pairs (Fig. 4a). Indeed, if phylogenetic relationships are ignored and one plots all 12 species averages for growth and herbivory together, the correlation between growth and herbivory disappears. Such an analysis treats each species' average for growth rate and defense as an independent data point, an assumption that is clearly not valid (Harvey and Pagel 1991).

Second, it allows one to make direct inferences about the phylogenetic patterns of plant defensive traits and how they relate to habitat specialization. For example, terpenes, phenolics, and leaf toughness in our genera exhibit strong signals of phylogenetic constraint. But, since species within those genera have a diverse group of defensive options, this apparent lack of evolutionary lability to completely turn on or off investment into a particular class of defense does not result in lineages becoming ecologically constrained to one particular soil type. For this reason, we observed no signal of phylogenetic constraint in protection effect (i.e., amount of herbivory) or growth in the genera. This is almost certainly due to the fact that the relevant traits that confer resistance to herbivores in low-resource habitats and faster growth in high-resource habitats are evolutionarily labile and involve quantitative increases and decreases of already-present qualitative traits related to growth and defense.

CONCLUSIONS

By manipulating the presence of herbivores, we discovered that defense differences interact with edaphic factors to restrict species to their specialized habitats. Although the potential for herbivore attack was similar in the two habitats, the impact of herbivory on growth and survivorship was much stronger in white-sand forest, giving solid evidence of strong selection for effective defense in white-sand forests. Measurements of defenses confirmed that white-sand specialists have a higher overall defense investment, although each genus expressed a different combination of defensive traits. These results confirmed theoretical predictions that species in low resource habitats evolve a higher optimal defense investment. By testing for defense and growth differences in white-sand and clay specialists within an explicit phylogenetic framework, our results strengthen the case that the trade-off between growth and defense is universal and governs patterns of plant distribution. This fundamental trade-off, mediated by herbivores, represents an important mechanism of plant coexistence that has been largely overlooked in studies of plant habitat specialization and niche assembly. Furthermore, this interaction between herbivory and resource heterogeneity should promote divergent selection in plant growth and defense strategies that increase the potential for ecological speciation.

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APPENDIX A

Detailed methods for the chemical analysis of terpenes, phenolics, and soluble protein (*Ecological Archives* E087-117-A1).

APPENDIX B

A table presenting all fixed-factor ANOVAs conducted on the growth and defense variables (*Ecological Archives* E087-117-A2).

APPENDIX C

A table presenting growth, herbivory, and defensive traits measured in the experiment for each species in the two soil types (*Ecological Archives* E087-117-A3).

APPENDIX D

Figures showing the effect of origin (white-sand vs. clay specialists) and the genus × origin interaction for (a) leaf growth, (b) height growth, (c) the effect of herbivory on leaf growth (protection effect), (d) protection effect on height growth, (e–j) chemical defenses, (k) leaf toughness, and (l) available foliar protein (*Ecological Archives* E087-117-A4).