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# Herbivores Promote Habitat Specialization by Trees in Amazonian Forests

Paul V. A. Fine,[1,2]* Italo Mesones,[3] Phyllis D. Coley[1]

In an edaphically heterogeneous area in the Peruvian Amazon, clay soils and nutrient-poor white sands each harbor distinctive plant communities. To determine whether a trade-off between growth and antiherbivore defense enforces habitat specialization on these two soil types, we conducted a reciprocal transplant study of seedlings of 20 species from six genera of phylogenetically independent pairs of edaphic specialist trees and manipulated the presence of herbivores. Clay specialist species grew significantly faster than white-sand specialists in both soil types when protected from herbivores. However, when unprotected, white-sand specialists dominated in white-sand forests and clay specialists dominated in clay forests. Therefore, habitat specialization in this system results from an interaction of herbivore pressure with soil type.

The floras of tropical forests in areas of high environmental heterogeneity have high beta diversity, leading many authors to conclude that habitat specialization in tropical plants contributes significantly to the global diversity gradient (1–4). The change in species composition between habitats (beta diversity) in plants is thought to be caused primarily by physiological adaptations to different abiotic conditions (5–7). But herbivores have been shown to affect plant population dynamics in many ways, including plant distributions (8–10). The best way to test the effect of herbivores on plant distributions is with transplant experiments involving multiple species, but few such studies have been conducted. Although they have evaluated only one or two species at a time, these studies have consistently shown that herbivores can significantly limit plant distributions, often restricting species to a subset of the habitats they could physiologically tolerate in the absence of herbivores (11–15). However, no such studies have been conducted in tropical forests [but see (16, 17)], where herbivore abundance and rates of herbivory far exceed those of the temperate zone (18). If herbivores interact with abiotic gradients to intensify differences in habitat quality, this phenomenon could account for the high degree of habitat specialization in environmentally heterogeneous areas within tropical systems.

The lowland Amazonian ever-wet rainforest in the Allpahuayo-Mishana Reserve near Iquitos, Peru (3°57'S, 73°26'W), provides an ideal system to study habitat specialization and the role of insect herbivores. Forests in the Iquitos area occur on a mosaic of soil types with well-defined boundaries, including extremely infertile white-sand soils adjacent to lateritic red-clay soils (19). These two soil types are each characterized by a highly distinctive but related flora (19–22). 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.

Janzen (23) argued that the most critical factors in the evolution of white-sand specialist plants were herbivores and plant defenses rather than physiological tolerance to poor soils. A mechanism that would produce such a result is a trade-off between allocations to growth and defense that selects for different traits in white-sand and clay forest species. We hypothesized that plants that do not invest sufficiently in defenses ought to be excluded from white-sand forests by herbivores, because the cost of replacing damaged tissue in such a nutrient-poor environment should be prohibitively high (24). In contrast, species that invest heavily in defenses should grow more slowly and be at a competitive disadvantage in clay forests.

[1]Department of Biology, University of Utah, 257 S. 1400 East, Salt Lake City, UT 84112, USA. [2]Environmental and Conservation Programs and Department of Botany, Field Museum of Natural History, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA. [3]Department of Forestry, Universidad Nacional de la Amazonía Peruana, Plaza Serafín Filomeno 246, Iquitos, Peru.

*To whom correspondence should be addressed. E-mail: fine@biology.utah.edu

**Fig. 1.** Phylogenetic relationships of the species in the transplant experiment with their family, genus, species, and soil type (origin). The phylogenetic tree is adapted from (37), and the Burseraceae phylogeny is adapted from (38). *Tetragastris panamensis* has been found to be imbedded within *Protium* sensu lato (38), therefore we are considering *Tetragastris panamensis* as a *Protium* clay specialist species. Soil type (origin) was determined by extensive tree and seedling inventories that we conducted in more than 25 sites throughout the 57,600-ha Allpahuayo-Mishana Reserve. Our designation of white-sand specialists is consistent with other inventories and a published flora from the area (19, 22, 38, 39). Our designation of clay specialists conforms to other species lists on clay soils in the area (19, 22, 38) as well as with lists from the Ecuadorian Amazon, which is almost entirely composed of clay soils and where white-sand forests are unknown (40).

| Family | Genus and species | Soil type (origin) |
|---|---|---|
| Annonaceae | *Oxandra xylopioides* Diels | Clay |
| Annonaceae | *Oxandra euneura* Diels | White sand |
| Burseraceae | *Tetragastris* * *panamensis* (Engler) Kuntze | Clay |
| Burseraceae | *Protium subserratum* (Engler) Engler | White sand |
| Burseraceae | *Protium nodulosum* Swart | Clay |
| Burseraceae | *Protium paniculatum* Engler | White sand |
| Burseraceae | *Protium opacum* Swart | Clay |
| Burseraceae | *Protium calanense* Cuatrec. | White sand |
| Burseraceae | *Protium trifoliolatum* Engler | Clay |
| Burseraceae | *Protium krukoffii* Swart | Clay |
| Burseraceae | *Protium heptaphyllum* subsp. *ulei* (Swart) Daly | White sand |
| Burseraceae | *Protium hebetatum* Daly | Clay |
| Malvaceae (Bombacoid) | *Pachira insignis* (Swartz) Swartz ex Savigny | Clay |
| Malvaceae (Bombacoid) | *Pachira brevipes* (Robyns) Alverson | White sand |
| Euphorbiaceae | *Mabea pulcherrima* Müll. Arg. | Clay |
| Euphorbiaceae | *Mabea subsessilis* Pax & Hoffmann | White sand |
| Fabaceae (Faboid) | *Swartzia arborescens* (Aublet) Pittier | Clay |
| Fabaceae (Faboid) | *Swartzia cardiosperma* Spruce ex Benth. | White sand |
| Fabaceae (Mimosoid) | *Parkia multijuga* Benth. | Clay |
| Fabaceae (Mimosoid) | *Parkia igneiflora* Ducke | White sand |

Using a 21-month reciprocal transplant experiment with 20 common white-sandand clay forest species from six genera, and manipulating the presence of herbivores, we directly assessed the effect of herbivory on the maintenance of habitat specialization in trees. We addressed three main questions: (i) Can soil specialists grow in a different edaphic environment, or is soil type alone a sufficient barrier for these plants? (ii) Do herbivores exclude clay specialists from white-sand forests? (iii) Do white-sand specialists grow more slowly than clay forest specialists, putting them at a competitive disadvantage in clay forests?

We transplanted 880 seedlings from six genera arranged in pairs of clay/white-sand specialist congeners (Fig. 1). In May 2001, we built 22 control and 22 herbivore exclosures (3 m by 3 m by 2 m) in clay and white-sand habitats, controlling for light levels (25). One seedling of each species was transplanted into each of the 22 controls and 22 exclosures, for a total of 20 seedlings per exclosure. The exclosures were completely covered by 1-mm fine nylon netting, whereas the controls had netting only on the roof. The experiment lasted until February 2003. To assess differences between seedlings in white-sand versus clay forests, and in control versus protected treatments (exclosures), we measured growth rate (leaf area per day and meristem height per day) and mortality (25).

Both clay and white-sand specialist species exhibited positive growth on both soil types. In white-sand habitat, clay specialists and white-sand specialists suffered statistically similar mortality when protected from herbivores (Fig. 2). These findings contradict the hypothesis that white-sand soil is probably toxic to nonadapted edaphic specialists because of differences in $H^+/Al$ ratios (26). Our results also contradict a related hypothesis that posits that the low nutrient availability and/or water retention capacity of white sand exclude most nonspecialist plants from white-sand forests (6, 27, 28). Our results showed that when protected from herbivores and planted in white-sand habitat, clay species produced more than twice the amount of leaf area [0.21 cm² day⁻¹ versus 0.09 cm² day⁻¹, $P < 0.05$ (Fig. 2A)] and grew significantly taller than white-sand species [0.09 mm day⁻¹ versus 0.05 mm day⁻¹, $P < 0.05$ (Fig. 2B)]. Summing the 18 months of measurements, protected clay specialists grew an average of 4.9 cm in height and 115.3 cm² in leaf area as compared to protected white-sand specialists' 2.7 cm in height and 49.4 cm² in leaf area. Although soil type does have a significant effect on seedling meristem growth rate ($P < 0.05$, table S3), clearly the restriction of clay specialist species to clay habitat cannot be explained solely as a function of soil type, at least not at the critical seedling stage.

Our results showed a strong effect of herbivores on the exclusion of clay specialist species from white-sand forests. When unprotected from herbivores in white-sand habitat, white-sand specialists exhibited a significantly higher leaf growth rate than clay specialists [0.15 cm² day⁻¹ versus −0.4 cm² day⁻¹, $P < 0.05$ (Fig. 2A)] and suffered only half the mortality rate [9% versus 20%, $P < 0.05$ (Fig. 2C and fig. S2)]. Taken together, the results from white-sand forests indicate that without the presence of herbivores, clay specialists survived just as well as white-sand specialists but grew taller and produced more leaf area and thus potentially could invade and displace white-sand specialists from white-sand forests.

Furthermore, white-sand specialist and clay specialist species responded differently to herbivore protection in both leaf and meristem growth rate [origin × protection interaction, $P < 0.0001$ and $P < 0.01$, respectively (tables S2 and S3)] and in mortality rate ($P < 0.05$) (Fig. 2). White-sand species did not respond to protection in either habitat ($P > 0.05$), presumably because their leaves are already highly defended against herbivores (23, 29). Indeed, chemical analyses of these seedlings showed that leaves of white-sand specialists contain significantly higher tannin: protein ratios than leaves of clay specialists (30). In contrast, clay specialist species gained a significant advantage from herbivore protection, producing 267% more leaf area per day when protected (Fig. 2A).

All species grew better on clay soils, but clay specialists outperformed white-sand specialists on clay soils, providing indirect evidence that interspecific competition limits the invasion of clay forests by white-sand species. In the clay soil, white-sand species exhibited significantly slower height ($P < 0.01$) and leaf ($P < 0.01$) growth than clay specialists [habitat × origin interaction (Fig. 2, A and B, and tables S2 and S3)]. These results are consistent with the hypothesis that there is a growth/defense trade-off in these tree seedlings and that the high defense levels of white-sand specialists preclude a rapid growth rate and place them at a competitive disadvantage in clay soils.

We conclude that herbivores play an important role in maintaining habitat specialization in clay and white-sand specialist trees. Although other factors certainly influence habitat specialization at other stages of a tree's life (such as seed predation, the ability to tolerate falling debris, root morphology, etc.), herbivores appear to have primary importance at the seedling stage, and in addition, likely continue to attack trees throughout a tree's life. Herbivores killed a significant percentage of tree seedlings in this system, and in white-sand forests they selectively attacked clay specialist transplants, maintaining the dominance of endemic white-sand specialists. Without herbivores, our results indicate that clay specialists outperform white-sand specialists in both soil types. However, with herbivores present, clay specialist species dominate only in clay forests and white-sand specialists dominate only in white-sand forests. The strong negative impact of herbivores on the growth and mortality rates of clay specialists underscores the intense selective pressure in white-sand forests favoring a large investment in antiherbivore defenses. Thus, the trade-off between growth rate and antiherbivore defense appears to restrict species to one habitat or the other for the six genera we studied, and habitat specialization results from an interaction of herbivore pressure with soil type.

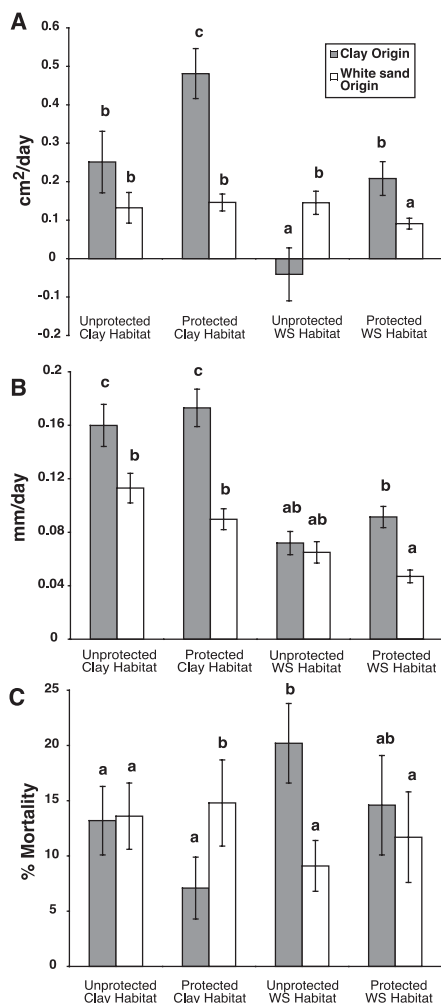Edaphic heterogeneity, especially in the tropics, has been implicated in the generation



**Fig. 2.** The effects of habitat and herbivore protection on (**A**) leaf area growth rate, (**B**) meristem height growth rate, and (**C**) percent mortality for white-sand and clay specialist species. Bars represent mean and ±1 SE. Values with different letters (a, b, and c) are significantly different from one another [Tukey tests for (A) and (B); Mann-Whitney $U$ for (C)].

and maintenance of high diversity (*1–4*, *20*, *31*, *32*), even though the specific mechanisms by which edaphic factors might influence diversity have, until now, never been tested (*33*). Our results suggest a mechanism by which herbivores may influence plant evolution: by accentuating habitat differences and thereby increasing the potential for edaphic heterogeneity to produce habitat specialization. There is mounting evidence that parapatric speciation across environmental gradients can occur in the face of gene flow (*34–36*). Thus, our data suggest that herbivores can be viewed as a diversifying force because they make existing abiotic gradients more divergent and cause finer-scale habitat specialization by magnifying the differences between habitats. Our study highlights the importance of edaphic differences, but the same mechanism could work across other abiotic gradients such as altitude, rainfall, and flooding regime—wherever the impact of herbivores is dissimilar across a habitat boundary.

### References and Notes

1. A. H. Gentry, *Ann. MO Bot. Gard.* **75**, 1 (1988).
2. D. B. Clark, M. W. Palmer, D. A. Clark, *Ecology* **80**, 2662 (1999).
3. H. Tuomisto *et al.*, *Science* **269**, 63 (1995).
4. H. Tuomisto, K. Ruokolainen, M. Yli-Halli, *Science* **299**, 241 (2003).
5. J. L. Harper, *The Population Biology of Plants* (Academic Press, New York, 1977).
6. D. A. Coomes, P. J. Grubb, *Vegetatio* **122**, 167 (1996).
7. A. Mark, J. Dickinson, J. Allen, R. Smith, C. West, *Austral Ecol.* **26**, 423 (2001).
8. S. Louda, K. H. Keeler, R. D. Holt, in *Perspectives in Plant Competition*, J. Grace, D. Tilman, Eds. (Academic Press, New York, 1990), pp. 413–444.
9. N. S. Cobb *et al.*, *Oecologia* **109**, 389 (1997).
10. H. Olff, M. Ritchie, *Trends Ecol. Evol.* **13**, 261 (1998).
11. M. Parker, R. Root, *Ecology* **62**, 1390 (1981).
12. S. M. Louda, *Ecol. Monogr.* **52**, 25 (1982).
13. S. M. Louda, *Ecology* **64**, 511 (1983).
14. S. M. Louda, J. Rodman, *J. Ecol.* **84**, 229 (1996).
15. C. D. G. Harley, *Ecology* **84**, 1477 (2003).
16. S. J. DeWalt, J. S. Denslow, K. Ickes, *Ecology* **85**, 471 (2004).
17. R. T. King, *Biotropica* **35**, 462 (2003).
18. P. D. Coley, J. A. Barone, *Annu. Rev. Ecol. Syst.* **27**, 305 (1996).
19. K. Ruokolainen, H. Tuomisto, in *Geoecologia y Desarrollo Amazonico: Estudio Integrado en la Zona de Iquitos, Peru*, R. Kalliola, S. Flores, Eds. (Univ. of Turku Press, Turku, Finland, 1998), pp. 253–368.
20. A. H. Gentry, in *Conservation Biology: The Science of Scarcity and Diversity*, M. Soulé, Ed. (Sinauer, Sunderland, MA, 1986), pp. 153–181.
21. K. Ruokolainen, A. Linna, H. Tuomisto, *J. Trop. Ecol.* **13**, 243 (1997).
22. R. Vásquez M., *Florula de las Reservas Biologicas de Iquitos, Peru* (Missouri Botanical Garden Press, St. Louis, MO, 1997).
23. D. H. Janzen, *Biotropica* **6**, 69 (1974).
24. P. D. Coley, J. P. Bryant, F. S. Chapin, *Science* **230**, 895 (1985).
25. Detailed information on materials and methods is available on *Science* Online.
26. J. Proctor, *Bot. J. Scotl.* **51**, 1 (1999).
27. E. Medina, E. Cuevas, in *Mineral Nutrients in Tropical Forest and Savanna Ecosystems*, J. Proctor, Ed. (Blackwell, Oxford, 1989), pp. 217–240.
28. J. A. Moran, M. G. Barker, A. J. Moran, P. Becker, S. M. Ross, *Biotropica* **32**, 2 (2000).
29. D. McKey, P. G. Waterman, N. Mbi, J. S. Gartlan, T. T. Struhsaker, *Science* **202**, 61 (1978).
30. P. V. A. Fine *et al.*, unpublished data.
31. K. Young, B. León, *Brittonia* **41**, 388 (1989).
32. R. Condit *et al.*, *Science* **299**, 666 (2002).
33. P. Sollins, *Ecology* **79**, 23 (1998).
34. J. A. Endler, *Geographic Variation, Speciation, and Clines* (Princeton Univ. Press, Princeton, NJ, 1977).
35. C. Moritz, J. Patton, C. Schneider, T. B. Smith, *Annu. Rev. Ecol. Syst.* **31**, 533 (2000).
36. R. Ogden, R. S. Thorpe, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 13612 (2002).
37. M. W. Chase *et al.*, *Ann. MO Bot. Gard.* **80**, 528 (1993).
38. P. V. A. Fine, D. C. Daly, G. Villa, I. Mesones, K. M. Cameron, unpublished data (available on request from the first author).
39. R. García-Villacorta, M. Ahuite R., M. Olórtegui Z., *Folia Amazónica* **14**, 11 (2002).
40. P. Jørgensen, S. Léon-Yánez, *Catalogue of the Vascular Plants of Ecuador* (Missouri Botanical Garden Press, St. Louis, MO, 1999).
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# Silencing the Jasmonate Cascade: Induced Plant Defenses and Insect Populations

**André Kessler, Rayko Halitschke, Ian T. Baldwin***

We transformed the native tobacco, *Nicotiana attenuata*, to silence its lipoxygenase, hydroperoxide lyase, and allene oxide synthase genes in order to inhibit oxylipin signaling, known to mediate the plant's direct and indirect defenses. When planted into native habitats, lipoxygenase-deficient plants were more vulnerable to *N. attenuata*'s adapted herbivores but also attracted novel herbivore species, which fed and reproduced successfully. In addition to highlighting the value of genetically silencing plants to study ecological interactions in nature, these results show that lipoxygenase-dependent signaling determines host selection for opportunistic herbivores and that induced defenses influence herbivore community composition.

The plant traits that are important for resistance to herbivore attack in nature are complex and operate on many spatial scales. They involve direct defenses (toxins, digestibility reducers, etc.) (*1*), which themselves protect plants, as well as indirect defenses, which work with components of a plant's community (natural enemies, diseases, etc.) (*2–6*). Moreover, plant defenses can be constitutively expressed or produced in response to an attacking pathogen or herbivore, when they are needed (*7*, *8*).

Phenotypic plastic responses such as herbivore-induced plant defenses are thought to be an adaptation to unpredictable environments (*8*). In native populations of *Nicotiana attenuata* in the southwestern United States, herbivory is an unpredictable selective factor. *N. attenuata*'s ephemeral occurrence after fires (*9*) forces its herbivore community to reestablish itself with every new plant population, and the plant produces a wide array of direct and indirect chemical defenses in response to this unpredictable herbivore attack (*10*). Many of the responses are specifically elicited by signals introduced into wounds during feeding (*11*), and most herbivore-induced responses studied to date require oxylipin signaling (*12*, *13*).

Genetic transformation provides a valuable tool with which to manipulate traits that mediate complex plant-herbivore interactions and allows an integrative analysis of single traits (*14–16*). However, transformants are usually evaluated with known challenges, not the vast number of unknowns that occur in nature. We used transformed lines of the wild tobacco species *N. attenuata*, which express *N. attenuata* lipoxygenase 3 (*NaLOX3*), hydroperoxide lyase (*NaHPL*), and allene oxide synthase (*NaAOS*) in an antisense orientation (as-*lox*, as-*hpl*, as-*aos,* respectively) (*17*) to study herbivore-induced plant responses in nature. All three enzymes are key regulators in two distinct oxylipin pathways (fig.

Department of Molecular Ecology, Max-Planck-Institute for Chemical Ecology, Hans-Knöll-Strasse 8, Jena 07745, Germany

*To whom correspondence should be addressed. E-mail: baldwin@ice.mpg.de