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THE CONTRIBUTION OF EDAPHIC HETEROGENEITY TO THE EVOLUTION AND DIVERSITY OF BURSERACEAE TREES IN THE WESTERN AMAZON

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Abstract.—Environmental heterogeneity in the tropics is thought to lead to specialization in plants and thereby contribute to the diversity of the tropical flora. We examine this idea with data on the habitat specificity of 35 western Amazonian species from the genera *Protium*, *Crepidospermum*, and *Tetragastris* in the monophyletic tribe Protieae (Burseraceae) mapped on a molecular-based phylogeny. We surveyed three edaphic habitats that occur throughout terra firme Amazonia: white-sand, clay, and terrace soils in eight forests across more than 2000 km in the western Amazon. Twenty-six of the 35 species were found to be associated with only one of three soil types, and no species was associated with all three habitats; this pattern of edaphic specialization was consistent across the entire region. Habitat association mapped onto the phylogenetic tree shows association with terrace soils to be the probable ancestral state in the group, with subsequent speciation events onto clay and white-sand soils. The repeated gain of clay association within the clade likely coincides with the emergence of large areas of clay soils in the Miocene deposited during the Andean uplift. Character optimizations revealed that soil association was not phylogenetically clustered for white-sand and clay specialists, suggesting repeated independent evolution of soil specificity is common within the Protieae. This phylogenetic analysis also showed that multiple cases of putative sister taxa with parapatric distributions differ in their edaphic associations, suggesting that edaphic heterogeneity was an important driver of speciation in the Protieae in the Amazon basin.

Key words.—Ecological speciation, habitat specialization, parapatric, *Protium*, tropical rainforest, white sand.

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Many authors have proposed that high habitat diversity contributes to high plant species richness in tropical rainforests (Gentry 1981, 1986, 1988; Tuomisto et al. 1995, 2003; Clark et al. 1998, 1999; Condit et al. 2002). Indeed, surveys have documented substantial turnover in tropical tree species composition across habitats and correlated these changes with various abiotic conditions (e.g., altitude, soil type or rainfall gradient; Gentry 1986, 1988; Tuomisto et al. 1995; Ruokolainen et al. 1997; Davies et al. 1998; Webb and Peart 2000; Pyke et al. 2001; Potts et al. 2002). Surveys alone, however, are not able to illuminate how environmental heterogeneity might contribute to diversification. The hypothesis that environmental heterogeneity actually leads to habitat specialization in plants has not been rigorously tested with data on the specificity of individual plant species to a particular habitat across an entire region, coupled with the phylogenetic relationships of habitat specialist trees in tropical rain forests. Such an approach is critical to test the causal connections of ecological patterns of habitat association to the evolutionary basis of specialization to a habitat (Ricklefs and Schluter 1993).

Here, we map habitat association onto a phylogeny of a diverse clade of Amazonian trees to evaluate the role of environmental heterogeneity in the origin of regional diversity for this group. If habitat specialization is not a driver of diversification in a clade, we expect to find either that most species will be associated with more than one habitat or that the habitat specialists among them of each habitat type are phylogenetically clustered (e.g., all white-sand specialists would be each other's closest relatives). In this latter case, the evidence would be consistent with a hypothesis that habitat specialization caused the initial splitting of lineages, but that the great majority of subsequent diversification events were caused by allopatric events via dispersal and/or vicariance. This result would be consistent with the idea that lineages are exhibiting phylogenetic niche conservatism (Wiens 2004; Wiens and Donoghue 2004). Conversely, if we find that habitat specialists have evolved repeatedly and independently, it would point to a more recent and active role for environmental heterogeneity in the diversification process. This would be consistent with the idea that ecological speciation plays an important role in diversification (Schluter 2000, 2001; Levin 2004).

We examine the contribution of habitat specialization to regional diversity in eight forests spanning more than 2000 km across the western Amazon and including three edaphic

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habitats found throughout terra firme in the Amazon basin: white-sand forests, terrace (also called brown-sand) forests, and clay forests. Plant species compositions are distinctive in each of these habitats throughout the tropics, as defined by greater overlap in species composition in plots located within each of the habitats than between them (Ashton 1976; Guillamet 1987; Tuomisto et al. 1995; Swaine 1996; Ruokolainen et al. 1997; Ruokolainen and Tuomisto 1998; Daly and Mitchell 2000; ter Steege et al. 2000a,b; Potts et al. 2002). A major component of this flora in the Amazon basin belongs to the tribe Protieae of the Burseraceae (Daly 1987; Ruokolainen and Tuomisto 1998; Pitman 2000). We therefore chose this monophyletic group to investigate edaphic specialization.

We first investigate to what degree each western Amazonian species of the Protieae could be classified as an edaphic specialist and which particular edaphic habitats it occupies. Next, we reconstructed a molecular-based phylogeny of these species and mapped habitat association onto the phylogeny to examine how many times habitat specialization has independently evolved in the clade.

MATERIALS AND METHODS

Study Organisms

The monophyly of the tribe Protieae Engl. consisting the genera *Protium* (about 120 species), *Crepidospermum* (five species), and *Tetragastris* (nine species) has been supported by phylogenetic analysis (Clarkson et al. 2002; Weeks et al. 2005). The morphological basis for separating these genera is weak (Daly 1987, 1989) so all three are included in this study. Species of *Crepidospermum* and *Tetragastris* are restricted to the Neotropics, while those of *Protium* are pantropical, with seven species recognized in the Paleotropics. We sampled every species encountered in our forest inventories (35 of the approximately 40 species of Protieae known to occur in the Western Amazonian lowlands). These include three species of *Crepidospermum*, one species of *Tetragastris*, and 31 of about 70 species of *Protium* known from the Amazon. In our phylogenetic study, we included one Central American species, one species from Madagascar, and three species from the Guianas. This effort encompassed all currently recognized sections of *Protium* (Daly 1987, 1989, 1992; Harley and Daly 1995).

Habitats

Terra firme forest in the Amazon basin cannot be considered a single homogenous habitat (e.g., Tuomisto et al. 1995). To examine habitat specificity for the Protieae, we surveyed three common edaphic habitats that have been reported to harbor distinct floras in the Amazon: white-sand forests, terrace forests, and clay forests (Anderson 1981; Guillamet 1987; Tuomisto et al. 1995; Ruokolainen et al. 1997; Ruokolainen and Tuomisto 1998; Daly and Mitchell 2000; ter Steege et al. 2000a,b). The different origins of white-sand, terrace formation and clay soils result from the complex geological history of the western Amazon that includes repeated marine incursions and massive tectonic events, the latter culminating in the rise of the Andes mountain range and leading to major changes in the direction of the main fluvial systems in the region (Räsänen et al. 1987; Hoorn 1993, 1994; Burnham and Graham 1999).

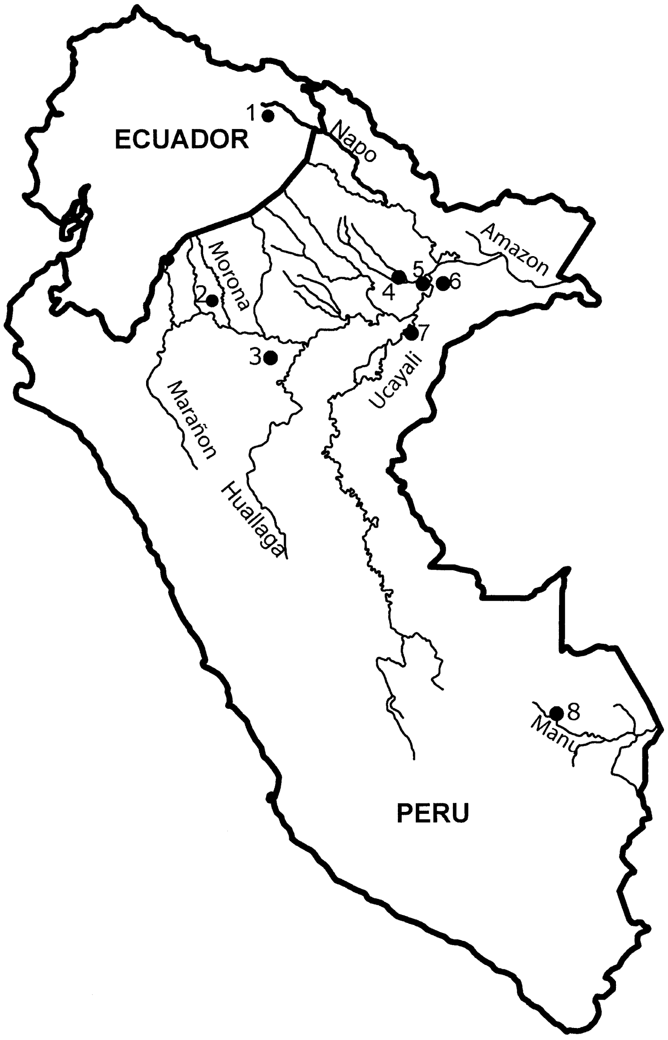
White sand is composed primarily of quartz, derived from eroded sandstone sediments of Precambrian origin that were deposited by rivers draining west prior to the Andean uplift. In the western Amazon, white-sand forests appear as small islands, rarely larger than a few square kilometers except in the Rio Negro basin, where they are reported to be quite extensive (Huber 1995). Overall, they comprise approximately 3% of the total area of the Amazon basin (ter Steege et al. 2000a). White-sand forest, also known as Amazonian caatinga, is perhaps floristically the most distinctive edaphic habitat in the Amazon, with many endemic species (Anderson 1981). Extreme nutrient poverty (Medina and Cuevas 1989; Coomes 1997; Coomes and Grubb 1998) causes the trees to have a stunted canopy relative to the other soil types, adding to the unique physical appearance of these forests.

Terrace formation derives from sands and gravels of Andean origin deposited by Pliocene and Pleistocene rivers after the Andean uplift (Hoorn 1993, 1994). These soils are sandy, mixed with some loam, silt, and clay. They are brown, orange, or yellow, and often are called ‘‘brown sand’’ soils in the literature (Tuomisto and Ruokolainen 1994; ter Steege et al. 2000a). Because terrace soils often include many types of sediments other than sand, we will refer to them as ‘‘terrace soils’’ throughout the text. Although recent in origin, there were probably large areas of the western Amazon covered by soils of similar appearance and fertility prior to Andean uplift (Struwe et al. 1997).

Clay soils (known as the Solimoes or Pebas formation) in the western Amazon originated from the erosion of Cretaceous metamorphic rocks that became exposed during the Andean uplift (Hoorn 1993). Sediment deposition was in swamps and shallow lakes, as well as in estuaries caused by Caribbean marine incursion as indicated by marine fossils and mangrove pollen found near Iquitos. Pebas formation clays occupy topographically lower areas, with the more recent Terrace soils having been deposited on top of them.

Forests Sampled

We sampled six forests in the Department of Loreto, northern Peruvian Amazon, and one in Yasuní National Park in the Ecuadorian Amazon (Fig. 1). Edaphic specialization among species of Protieae was intensively studied at the Allpahuayo-Mishana National Reserve, southwest of Iquitos, Peru (Fig. 2). This reserve comprises mostly terra firme forests that are a mosaic of white-sand, terrace, and clay habitats (Vásquez 1997). We also visited six other forests in the northwestern Amazon to investigate whether the edaphic associations found among species in Allpahuayo-Mishana were congruent with habitats hundreds of kilometers distant. In all of the above forests, we collected all Burseraceae trees that were encountered, and voucher specimens of all species are deposited in the herbaria of the New York Botanical Garden (NY), and/or the Field Museum of Natural History (F) and the Universidad Nacional de la Amazonía Peruana (AMAZ) (see Appendix available online only at http://dx.doi.org/10.1554/04-745.1.s1). To add to our data on habitat associ-



Site	Latitude/Longitude	Habitats Surveyed
1. Yasuní	01° S 76° W	Clay
2. Tierra Blanca	04° 16' S 77° 14 W	Terrace/White Sand
3. Jeberos	05° 18' S 76° 16' W	Terrace/White Sand
4. Alta Nanay	03° 43' S 74° 07' W	Terrace/White Sand
5. Allpahuayo-Mishana	03° 57' S 73° 24' W	Clay/Terrace/White Sand
6. Tamshiyacu	03° 59' S 73° 04' W	Clay/Terrace/White Sand
7. Jenaro Herrera	04° 51' S 73° 36' W	Terrace/White Sand
8. Manu	12° S 71° W	Clay

FIG. 1. Map of the eight forests inventoried in Peru and Ecuador, including the latitude and longitude and the edaphic habitats present at each site.

ation in the Protieae in the western Amazon, Nigel Pitman contributed a species list of Protieae from his taxonomically standardized network of 13 1-ha plots established by John Terborgh, Percy Nuñez, and others in terra firme forest in and around Manu National Park in southeastern Peru.

Habitat Specificity and Distribution of the Protieae

Habitat associations of Protieae with white-sand, terrace, and clay forests were determined in 67 plots at 18 different sites within Allpahuayo-Mishana for each of the three habitats (28 plots, nine sites in white sand; 15 plots, five sites in brown sand; and 24 plots, six sites in clay). Each site included a maximum of four plots. Sites were never less than 100 m from one another, and the 18 sites were distributed within an area of 1500 ha (Fig. 2). Plots were 1000 m^2 in size, and either 20 × 50 m rectangles (all white-sand and terrace plot, half of the clay plots) or 200 × 5 m belts (as half of the clay sites occur as strands along streams). In each plot, all individuals above 0.5 m in height from the genera *Protium*, *Crepidospermum*, and *Tetragastris* were tagged, identified, and collected if necessary. Representatives from each species were collected as vouchers for DNA extraction.

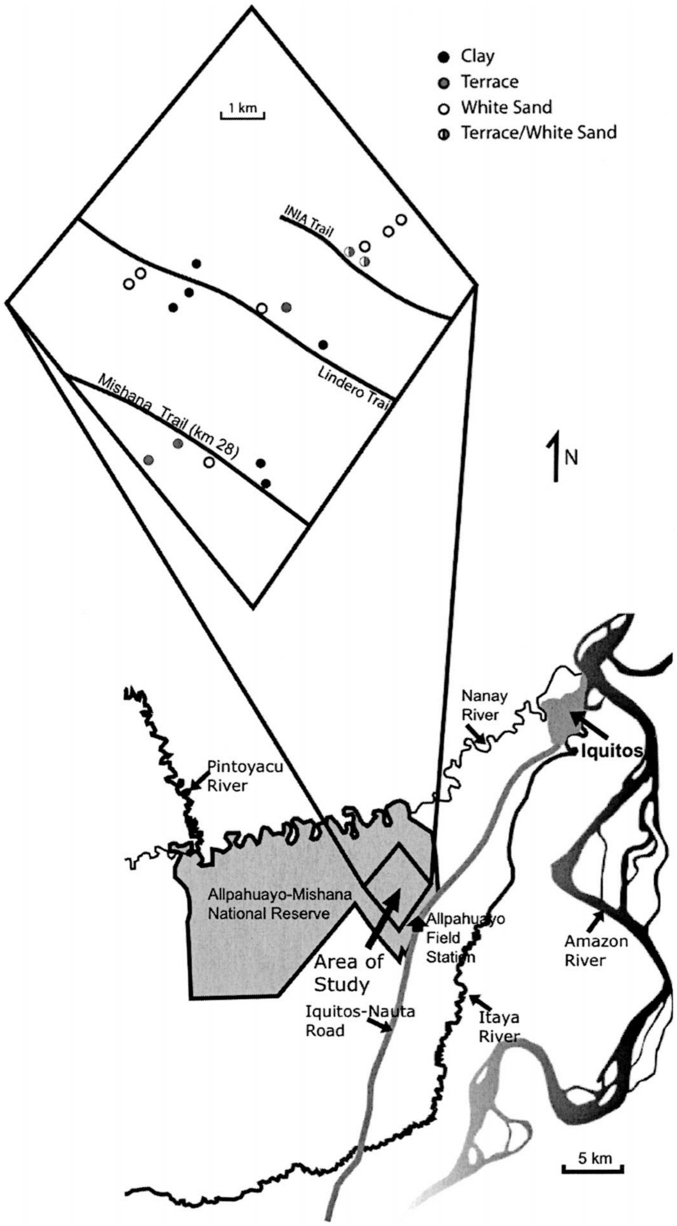


FIG. 2. Map of the Allphuayo-Mishana National Reserve near Iquitos, Peru. The diamond details the area of the 18 sites for Burseraceae inventories. Black, gray, and white circles represent sites that are on clay, terrace, and white-sand soils, respectively.

To characterize the degree of habitat specificity for each species, we used Fisher's exact tests on each species' presence/absence data for all plots. By testing presence/absence at the plot level, we avoided the problem that trees often occur in spatially nonindependent patterns within a plot (Clark et al. 1999; Plotkin et al. 2000; Harms et al. 2001); in addition Fisher's exact test is highly conservative for small sample sizes (Zar 1999). We were thus able to include all but the rarest four species in the statistical analysis. Each species was tested for association with each habitat type, and each Fisher's exact test gives significance values for both association and nonassociation to each particular habitat. Habitat associations observed in the Allpahuayo-Mishana Reserve were also examined in seven other forests and we collected vouchers of all Burseraceae species in each habitat type for each forest to determine if habitat specificity remained constant across each species' range. In addition, with the additional sampling, we were able designate habitat associations for the four rare species in Allpahuayo-Mishana. In Yasuní National Park, Ecuador, the third author collected a voucher in silica gel for each species of Protieae encountered at the Estación Científica Yasuní.

For each forest sampled (except Yasuní and Manu), we noted the color and texture of soils in 1-m pits and measured the depth of the root mat. The forests at both Yasuní and Manu have previously been described as occurring on clay soils (Foster 1990; Huston 1994; Pitman 2000; Pitman et al. 2001). In Allpahuayo-Mishana, to test for differences in nitrogen availability among the three edaphic habitats, we set out 50 ion-exchange resin bags in the 18 sites. Nylon stocking bags filled with 8 g of Rexyn 300 (H-OH; Fisher Scientific, Fairlawn, NJ) analytical grade resin beads were tied at both ends with cable ties. In May 2002, we placed the bags beneath the litter layer and root mat at the organic material–mineral soil interface (Binkley and Matson 1983). We collected the bags after five weeks, and they were extracted with KCl and measured by standard techniques with an autoanalyzer (University of Wisconsin Soils Laboratory). Nitrate, ammonium, and litter depth differences were tested for significance between soil types with a Kruskal-Wallis nonparametric ANOVA.

Phylogenetic Study

Genes used in analyses and sequencing methods

The internal transcribed spacer region (ITS) and the external transcribed spacer region (ETS) of nuclear ribosomal DNA repeat region were sequenced for phylogenetic analysis. We were able to sequence 35 species for ITS sequences (AY375490–AY375527) and 25 species for ETS sequences (AY964604–AY964636). The combined analysis included 40 species; of these, 30 were sequenced for both ITS and ETS, eight yielded sequences only for ITS, and two yielded sequences only for ETS (see online Appendix).

For 12 of the 40 species (listed in Appendix), between two and four specimens were sequenced per species. Specimens identified as the same species were indeed each others closest relatives and the accessions always differed by 0–1% uncorrected sequence divergence for ITS or ETS. Therefore, for the combined analyses, only one accession per species was used to reduce the analysis time.

We used three species of *Bursera* as outgroups, because *Bursera* was identified as among the closest relatives of *Protium*, *Crepidospermum*, and *Tetragastris* in a recent phylogenetic analysis (Weeks et al. 2005). We downloaded DNA sequences of *Bursera* for ITS and ETS from GenBank (AF080024, AF080026, AF080030) as well as an ETS sequence published in Weeks (2003) to include them in our sequence alignment and phylogenetic analysis.

Total genomic DNA was extracted using the FastPrep (Qbiogene, Inc., Carlsbad, CA) and glassmilk method from approximately 0.5 cm^2 dried leaf tissue. Fragments of nuclear DNA were amplified using four primers for ITS and two primers for ETS. The ETS primers' locations in the ETS region are described in Weeks et al. (2005). The four ITS primers included one reverse primer located in the 26S subunit, amplifying across the ITS 2 region (ny43: TAT GCT TAA AYT CAG CGGCT), one forward primer located in the 18S subunit amplifying across the ITS 1 region (ny183: CCT TAT CAT TTA GAG GAA GGAG), and two primers located within the 5.8S subunit, one forward primer amplifying the ITS 1 region (ny45: GCA TCG ATG AAG AAC GTAGC), and one reverse primer amplifying the ITS 2 region (ny109: GTG ACG CCC AGG CAG ACGT). ITS and ETS regions were amplified and cycle-sequenced using standard methods (with cleaning of sequencing products using Sephadex columns; Sigma-Aldrich, St. Louis, MO), and run on an ABI 377XL DNA sequencer (Applied Biosystems, Foster City, CA). The resulting electropherograms were edited using Sequencher 3.1 (GeneCode Corp., Ann Arbor, MI). The complete matrix was initially aligned using Sequencher and adjusted by eye using MacClade 4.05 (Maddison and Maddison 2001).

Parsimony and Bayesian analyses

A partition homogeneity test was performed as implemented in PAUP* 4.0b10 with 100 replicates to confirm the different nuclear genes sequenced (ITS and ETS) could be combined for phylogenetic analysis (Farris et al. 1995). The combined ITS/ETS data was first analyzed using the parsimony criterion in PAUP* version 4.0b10 (Swofford 2002) with gaps treated as missing data and characters weighted equally. Equally parsimonious trees were found by executing a heuristic search with 1000 random addition replicates and TBR branch swapping. Character support for the relationships were estimated by bootstrap analyses of 100 heuristic search replicates using the TBR branch swapping algorithm.

The likelihood-ratio test was used in MrModeltest (Nylander 2004), a simplified version of Modeltest (Posada and Crandall 1998) designed for Bayesian analysis to select the best model of molecular evolution with the fewest parameters that fit the dataset. These parameter estimates were used in all subsequent Bayesian analyses. We combined the ETS and ITS datasets and, using a mixed model, analyzed the substitution parameters separately for each gene partition. Bayesian inference of phylogeny was performed using MRBAYES version 3.0b (Huelsenbeck and Ronquist 2001) with the following specifications: (1) assuming a general time-reversible model of nucleotide substitution with estimated base frequencies, proportion of invariable sites, and rates for variable sites following a gamma distribution as selected by MrModeltest; and (2) running the Markov chain for 5×10^6 generations, sampling one tree every 100 generations. Following the suggestions outlined in Huelsenbeck and Ronquist (2001), we graphed the likelihood scores of all generations, and then discarded the first 1×10^6 generations as ''burn-in.'' PAUP* 4.0b10 was used to compute a 50% majority-rule consensus tree of the retained trees.

Evolution of soil association

Habitat association in plants results from a complex set of correlated characters, all of which together can be considered an extrinsic trait of a species (cf. Webb et al. 2002). For each species, soil association was scored as a discrete character: white-sand (W), terrace (T), clay (C), or any combination of the three. The number of changes was mapped onto the strict consensus tree of the 55 most parsimonious trees. Character reconstructions assumed Fitch parsimony and accelerated transformation optimization (ACCTRAN).

To look at the direction of evolutionary change among edaphic habitat types, we counted the changes (gains and losses) between each edaphic habitat in the 55 most parsimonious trees. Equivocal cycling was performed in MacClade 4.05 to examine all most-parsimonious reconstructions and to count the number of changes (Maddison and Maddison 2001). For polymorphic terminal taxa (taxa associated with more than one soil type), changes from the resolved state at the terminal node to each of the observed states were counted in the terminal taxon (Maddison and Maddison 2001).

We also evaluated soil specialization in a Bayesian framework with stochastic mapping (Nielsen 2002; Huelsenbeck et al. 2003). Similar to parsimony-based ancestral state reconstruction, stochastic mapping estimates the history of character changes on a phylogeny. Stochastic mapping simulates character evolution using an explicit transformation model, and unlike parsimony does not tend to underestimate the actual number of changes that occurred. We scored each species as associated (1) or not associated (0) with white-sand, terrace, and clay soils, with each soil type treated as a separate character. To account for uncertainty in the phylogeny of Protieae, character histories were mapped over the 40,000 trees sampled from the posterior density by the combined Bayesian mixed-model analysis. In the simulations, the bias parameter Π_0 was drawn from a uniform prior distribution, and branch lengths were scaled to yield a total tree length of one.

RESULTS

Habitat Specificity in Allpahuayo-Mishana

We encountered 1158 individuals from 29 different tree species of *Crepidospermum*, *Protium*, and *Tetragastris* in our 67 plots in Allpahuayo-Mishana (Table 1). Twenty-five species occurred in a sufficient number of plots to evaluate with Fisher's exact tests. Of these 25 species, 21 were significantly associated with only one habitat, three were associated with two habitats, and one was significantly nonassociated with one habitat (associating with the other two by default). Of the habitat specialists, one species was a white-sand specialist, 10 were terrace specialists, and 10 were clay specialists. Two of the four nonspecialists were associated with clay and terrace soils and two were associated with white-sand and terrace soils. Although three species were encountered in white-sand, terrace, and clay plots, all three of these species were significantly unassociated with at least one of these habitat types. In addition, mature individuals of these three species were never encountered in plots of the nonassociated habitat (Table 2). Thus, no species was classified as a complete generalist (associated with all three habitats), and no species was associated with the combination clay and white-sand habitat.

TABLE 1. Habitat associations (n, no. of plots) in Allpahuayo-Mishana. Numbers in bold (the numbers of plots a species was collected in) indicate significant positive associations with a habitat type (Fisher's exact test), and asterisks indicate significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Asterisks without boldface indicate significant nonassociations.

	Habitat					
Species	White sand (n = 28)	Terrace (n = 15)	Clay (n = 14)	Total plots (n = 67)	Total trees (n = 1157)	Soil association
Protium heptaphyllum ssp. *ulei*	**10*** **	0	0**	10	75	W
Protium rubrum	1	0	0	1	1	rare
Protium calanense	6	4	0**	10	47	(W/T)
Protium subserratum ssp. *subserratum*	**20***	**12***	4***	36	225	W/T
Crepidospermum pranceii	0	**3** **	0	3	3	T
Protium altsonii	0*	**4***	3	7	8	T
Protium crassipetalum	0***	**11*** **	2	13	45	T
Protium decandrum	0	**4** **	0	4	16	T
Protium divaricatum ssp. *krukoffii*	0	**4** **	0	4	8	T
Protium ferrugineum	3*	**10*** **	6	19	40	T
Protium gallosum	0	**3** **	0	3	5	T
Protium grandifolium	0	**3***	1	4	15	T
Protium klugii	1*	**9*** **	1*	11	34	T
Protium pallidum	0	2	2	4	9	rare
Protium paniculatum	4	**10*** **	0***	14	111	T
Protium amazonicum	0***	**8***	**13** **	21	46	T/C
Protium opacum	0***	**10***	**17*** **	27	144	T/C
Protium divaricatum ssp. *divaricatum*	0	1	3	4	4	rare
Protium guacayanum	0***	7	**11***	18	52	C
Protium hebetatum	0**	3	**6***	9	16	C
Protium trifoliolatum	0***	2	**11*** **	13	20	C
Crepidospermum goudotianum	0**	0	**8*** **	8	19	C
Crepidospermum rhoifolium	0	0	**5** **	5	5	C
Protium glabrescens	0	0	**5** **	5	13	C
Protium nodulosum	0***	3	**24*** **	27	207	C
Protium krukoffii	0***	3	**17*** **	20	57	C
Protium sagotianum	0***	0*	**13*** **	13	15	C
Protium tenuifolium	0	0	1	1	4	rare
Tetragastris panamensis	0*	0	**6** **	6	11	C

Habitat Specificity in the Western Amazon

Overall, including Allpahuayo-Mishana and all of the other forests surveyed in the western Amazon, we collected 35 total species of *Crepidospermum*, *Protium*, and *Tetragastris*, adding six new species to our total from Allpahuayo-Mishana (Table 2). Comparing the results from Allpahuayo-Mishana to the other seven western Amazonian forests in Figure 1, 21 of the 25 species that also occurred in the other forests were collected in the same habitats that they were statistically associated with in Allpahuayo-Mishana. Four of these species were encountered in additional habitats, although three of them, (*Protium hebetatum*, *P. trifoliolatum*, and *P. guacayanum*) were also found in terrace habitat in Allpahuayo-Mishana but were not significantly associated with it. We then added terrace association to these species for character-scoring (Table 1). The fourth species (*P. opacum*) was collected in two different white-sand forests. In each case only a single individual was collected, and *P. opacum* was not designated as associated with white-sand since these two collections represented less than 1% of our observations of *P. opacum* from clay and terrace soils. The four rare species from Allpahuayo-Mishana that could not be evaluated with Fisher's exact tests were recorded in the other western Amazonian forests and in the same edaphic habitats in which they were encountered in Allpahuayo-Mishana. We thus used this additional evidence to designate soil associations for these species.

Overall, we found a total of 35 species of Protieae, of which 26 were designated specialists on one soil type (74%): two white-sand specialists, 14 terrace specialists, and 10 clay specialists. There were three terrace/white-sand associated species, six terrace/clay associated species, no clay/white-sand associated species, and no species that was associated with all three soil types. In summary, almost three-quarters of the species were associated with only one habitat, with the remainder associated with (or found in) two of the three habitats, and not a single species that was a statistically significant generalist. Most species of Protieae are habitat specialists as defined here.

Habitat Characteristics of Allpahuayo-Mishana

Litter depth, nitrate, and ammonia all differed significantly among white sand, terrace and clay habitats (Table 3). White-sand sites were the most nitrogen deficient, the clay sites were the least deficient, and the terrace plots sites intermediate.

Phylogenetic Analyses

The maximum parsimony analysis resulted in 55 most parsimonious trees of 857 steps (CI = 0.608, RI = 0.691). Of 1159 total characters, 385 were variable and 215 were parsimony informative. The Bayesian posterior probabilities were mapped onto a majority rule consensus of all 40,000

TABLE 2. Checklist of Protieae species from the eight forests included in the study. The abbreviations correspond to the sites marked in Figure 1. Xs in boldface denote trees larger than 4 m. Underlined Xs are species that either were collected with fruits or flowers, or with large seedling carpets beneath them. Xs in parentheses are species from Allpahuayo-Mishana that were collected in a habitat, but were not significantly associated with that habitat (see Table 1). The soil association column indicates how the species was scored for character reconstructions (Figs. 3, 4). Collection numbers and accession numbers for each species are provided in the Appendix.

Species	Soil association	White-sand sites						Terrace sites						Clay sites			
		A-M	JH	Tam.	Jeb.	TB	AN	A-M	JH	Tam.	Jeb.	TB	AN	A-M	Tam.	Yasuní	Manu
Protium rubrum	W	**X**		**X**													
Protium heptaphyllum ssp. *ulei*	W	**X̲**			**X̲**												
Protium calanense	WT	**X̲**	**X**	X				**X**	X		**X**	X	X				
Protium laxiflorum	WT		**X**	X	**X̲**	X	X				**X**						
Protium subserratum ssp. *subserratum*	WT	**X̲**	**X**	**X**	**X**		**X**	**X**	X	X	X	**X**	X	(X)			
Crepidospermum pranceii	T							**X**	X								
Protium altsonii	T							(X)	X					(X)			
Protium apiculatum	T										**X**						
Protium crassipetalum	T	(X)						**X**		X	X	X	X	(X)			
Protium decandrum	T							**X̲**									
Protium divaricatum ssp. *krukoffii*	T							**X̲**									
Protium elegans	T										**X**	X					
Protium ferrugineum	T	(X)						**X̲**	X	**X̲**	X	X	X	(X)	X		
Protium gallosum	T							**X**	X	X							
Protium grandifolium	T							**X**			X			(X)			
Protium klugii	T	(X)						**X̲**						(X)			
Protium pallidum	T							(**X**)			X		X	(X)			
Protium paniculatum	T	(X)						**X̲**	X		X		X				
Protium urophyllidium	T								X	X							
Protium amazonicum	TC							**X̲**			X			**X**	X	**X**	**X**
Protium divaricatum ssp. *divaricatum*	TC							(X)			X	X		(**X**)			
Protium guacayanum	TC							(X)	X	X		X		**X̲**			
Protium hebetatum	TC							(**X̲**)	X	X	X	**X**		**X̲**	X		
Protium opacum	TC		**X**			X		**X**	X					**X̲**	X		
Protium trifoliolatum	TC							(X)			X		X	**X̲**	X	**X**	
Crepidospermum goudotianum	C													**X**			
Crepidospermum rhoifolium	C													**X**	X	**X**	
Protium aidanum	C															**X**	
Protium aracouchini	C															**X**	
Protium glabrescens	C													**X**		**X**	
Protium nodulosum	C							(X)						**X̲**	X	**X**	
Protium krukoffii	C							(X)						**X̲**	X		**X**
Protium sagotianum	C													**X**		**X**	**X**
Protium tenuifolium	C													**X**		**X**	**X**
Tetragastris panamensis	C													**X̲**		**X**	**X**

TABLE 3. Differences in physical characteristics and nitrogen availability between the white-sand, terrace, and clay forest sites. Nitrogen (ammonium, nitrate, and total N) numbers are expressed in parts per million (ppm) and are presented with their mean and ±1 standard error. Significance between sites was determined with Kruskall-Wallis tests; letter superscripts that are different indicate means that are significantly different from one another.

Variable	White-sand sites	Terrace sites	Clay sites	Significance
Soil colors (0–100 cm depth)	white, whitish gray	brown, orange, yellow	red, gray, brown, purple	
Soil texture (0–100 cm depth)	sand (poorly drained sites have white sand atop peaty hardpan)	sand, silt, loam	clay	
Root mat depth (cm)	8.5 ± 0.7^a	3.6 ± 0.6^b	1.0 ± 0.4^c	$P < 0.0001$
NH_4^+ availability (ppm)	62.1 ± 18.7^a	147.0 ± 33.7^b	135.2 ± 21.8^b	$P < 0.01$
NO_3^- availability (ppm)	25.6 ± 12.1^a	126.1 ± 21.8^b	349.2 ± 35.3^c	$P < 0.0001$
Total N availability (ppm)	87.7 ± 22.5^a	273.2 ± 48.5^b	484.4 ± 46.3^c	$P < 0.0001$

burn-in trees (Fig. 3). Congruence in phylogenetic signal between the two methods of analysis is very high, with only minor changes in the overall topologies depending on the type of analysis and no changes in well-supported clades (cf. Figs. 3 and 4). Bootstrap values from the parsimony analysis are mapped together with the posterior probabilities onto the Bayesian topology (Fig. 3). Posterior probabilities are consistently slightly higher than bootstrap probabilities, similar to two recent studies that have explored both methods (Reed et al. 2002; Wilcox et al. 2002).

The combined ETS-ITS analysis had a very similar topology to ETS alone and ITS alone (not shown). The main difference is in the slightly lower support values present in the combined analysis for some clades compared to the ITS analysis alone. This is most likely due to the presence of taxa with only ITS or ETS in the dataset (see online Appendix). However, we propose that the inclusion of a greater number of taxa in the combined analysis provides a more robust test of our hypotheses.

Character Optimization and Direction of Change

Mapping all the soil associations on the strict consensus tree in MacClade 4.05 resulted in a tree with a minimum of 10 and a maximum of 16 steps (Fig. 4). White-sand association was gained five times, clay association was gained a minimum of six times, and terrace association was gained a minimum of four times (Fig. 4). Terrace habitat was optimized as the ancestral condition for the Protieae in all of the 55 most parsimonious trees (Fig. 4). The matrix of changes (gains and losses) from one edaphic condition to another is listed in Table 4. The general trend is of the loss of terrace association and gain of clay and white-sand association.

The stochastic mapping results qualitatively match the parsimony-based character optimizations, the main difference being attributable to inclusion of all trees (excluding the burn-in trees), while the parsimony optimizations were realized with only the 55 most parsimonious trees. Table 5 shows the probabilities of each number of gains of soil association for each soil type. The stochastic mapping showed clear results for white-sand association. White-sand association evolved five times independently for more than 96% of the entire set of 40,000 trees, consistent with the parsimony-based optimization (Fig. 4). For clay and terrace association, stochastic mapping yields no clear resolution for the number of gains of soil association that have occurred across the entire dataset. This is likely a reflection of the uncertainty in the topology of the trees, especially along the spine (Fig. 3). Nevertheless, the cumulative probability for both zero or one gains for clay associations is less than 5%, with the highest probabilities being between two and 11 gains. Terrace association showed the highest probabilities between one and eight gains, and the large proportion of trees with low numbers of terrace gains is consistent with the hypothesis that terrace association is the ancestral condition (Fig. 4).

DISCUSSION

Most Species of Protieae Are Habitat Specialists

Almost three-quarters of the 35 species of Protieae sampled in this study were associated with only one soil type. Whereas published floras and florulas from the Amazon rarely differentiate between terra firme habitats, thus making it impossible to compare the habitat specificity data to them, they list many Protieae species as being associated with different habitats including white-sand forests (including *Protium heptaphyllum* subsp. *ulei* and *P. subserratum*), tepuis, cloud forests, gallery forests in savannas, and flooded habitats (Daly 1997; Vásquez 1997; Ribeiro et al. 1999). Our survey data indicate that in the western Amazon, edaphic heterogeneity combined with nonrandom species-habitat associations contributes to high diversity of Protieae species. For example, an area with only one terra firme soil type, such as Yasuní National Park (which has been reported as amongst the highest tree alpha-diversity sites in the Neotropics; Pitman 2000; Pitman et al. 2001) has less than half the total number of Protieae species compared to Allpahuayo-Mishana (Table 2).

We found that the edaphic specialization patterns documented at Allpahuayo-Mishana were highly congruent with forests hundreds and even thousands of kilometers distant (Table 2). Burseraceae trees are generally dispersed by birds, allowing for wide distribution for many species (Daly 1987). Even white-sand specialist taxa like *P. heptaphyllum* subsp. *ulei* grow in white-sand habitat islands throughout much of the Amazon basin (Daly 1987, 1997). While dispersal limitation may be relevant in understanding the changes in species composition over distances for other groups of tropical trees (Hubbell et al. 1999), our data indicate that dispersal limitation plays no role in determining the large-scale ecological patterns of habitat specialization in the Protieae.

Other studies on a variety of scales throughout the tropics

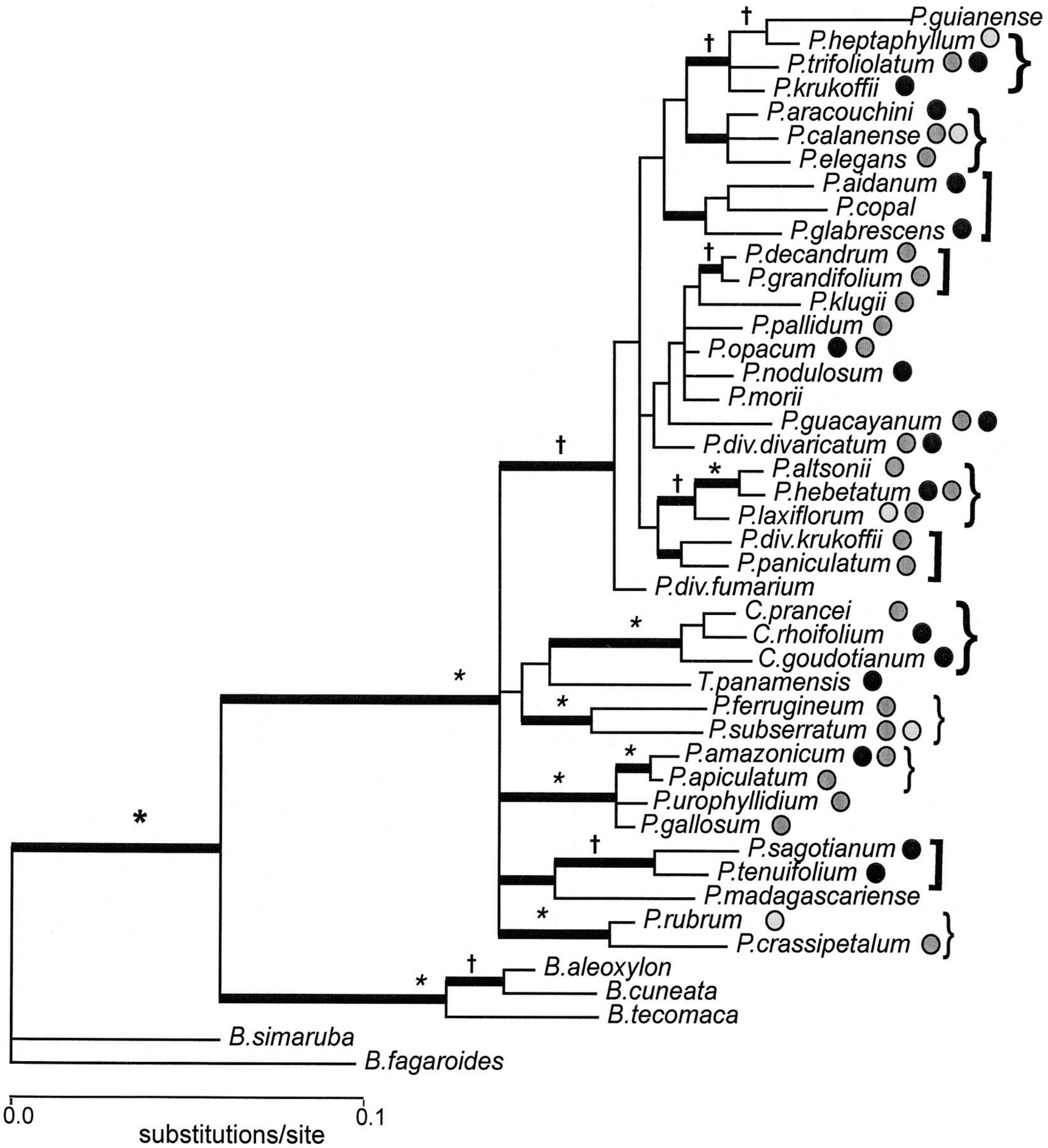


FIG. 3. Majority-rule consensus of all Bayesian trees. Posterior probabilities of 95% and higher are noted with bold branches. Maximum parsimony bootstrap values are mapped onto the same nodes, * for greater than 95, and † for greater than 80 bootstrap support. Black, gray, and white circles represent clay, terrace, and white-sand habitat associations, respectively. Curly bracketed sister groups include more than one type of edaphic specialist, while square bracketed sister groups include taxa with the same edaphic association. The generic names for the taxa are abbreviated, see Table 1 for complete species and genus names, and Appendix (available online only) for species authors.

have documented changes in plant species compositions between white sand, terrace, and clay soil sites. Duivenvoorden (1995) found floristic similarity was higher among white-sand plots than between white-sand and clay and terrace plots that covered more than 200 km along the Rio Caquetá in Colombia. ter Steege et al. (2000a) tested a network of 51 1-ha plots throughout Guyana for habitat associations between white-sand, brown-sand (analogous to terrace soils), and clay sites. They found about half of the 238 species had significant associations with either white-sand or brown-sand soils (clay sites were not included in this analysis). Potts et al. (2002) tested species turnover in 12 sites located in a 500 × 150 km area of Borneo and found significant differences between clay and sandy sites. While many species were encountered on only a single plot, for the 60 that were collected in multiple plots, 72% of them were restricted to only sand or clay habitat. Studies evaluating species turnover in ferns, Melastomataceae, and trees in seven sites located in a 200 × 300 km area in the western Amazon found that floristic similarity patterns of the seven sites are grouped into three clusters that correspond to white sand, terrace, and clay habitats (Tuomisto et al. 1995; Ruokolainen and Tuomisto 1998). Finally, several small-scale studies have compared lists of species on clay, white-sand, or terrace sites and found little or no overlap among habitats for ferns, Melastomataceae, and Lauraceae (Young and León 1989; van der Werff 1992;

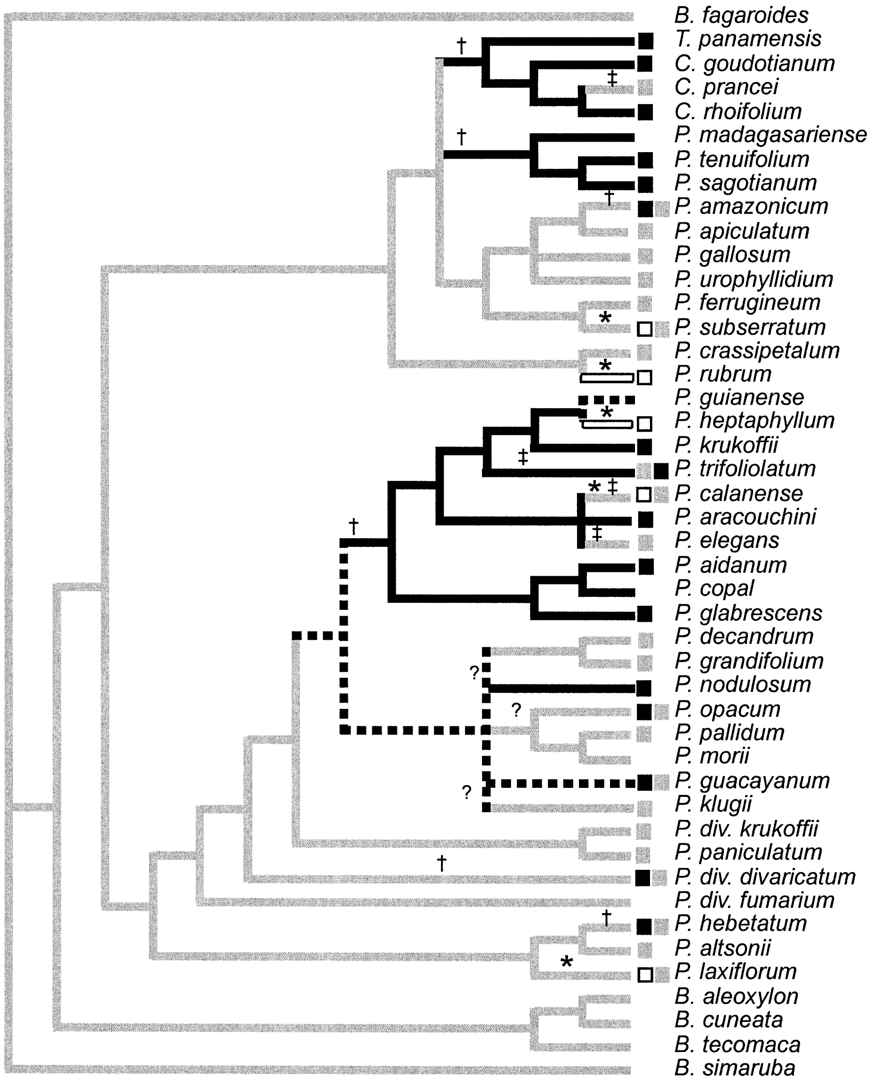


FIG. 4. Parsimony-based character optimizations for white-sand, terrace, and clay associations mapped onto the strict consensus tree from the 55 most parsimonious trees. White-sand is represented with white, terrace by gray, and clay by black lines and boxes. Uncertain ancestral state is represented by a dotted line, * represent unambiguous gains of white-sand association, ‡ represent unambiguous gains of terrace association, † represent gains of unambiguous clay association, and ? represent gains by either clay or terrace (either are possible depending on the resolution of the uncertainty in the ancestral state reconstruction).

Tuomisto and Ruokolainen 1994). This breadth of sites and of taxonomic groups suggests that habitat specialization to white-sand, clay and terrace soils is a pattern common to tropical plant groups and is not confined to Burseraceae trees.

TABLE 4. Matrix showing the minimum and maximum number of changes from clay, terrace, and white-sand soil associations to different associations computed over all 55 most parsimonious trees with equivocal cycling.

		To		
		Clay	Terrace	White sand
From	Clay	—	1–5	0–2
	Terrace	8–12	—	3–5
	White sand	0–1	0–1	—

The Ancestral Soil Association in the Protieae and Its Relation to the Geologic Histories of White-Sand, Terrace, and Clay Soils in the Western Amazon

Parsimony-based and Bayesian character optimizations are both consistent with the hypothesis that terrace soil association was the ancestral state in the Protieae (Fig. 4, Table 5). In addition, equivocal cycling results suggest that terrace soil association was lost eight to 13 times and only gained one to six times (Table 4). However, the fact that there are many taxa missing from our analysis (from areas outside of the western Amazon) means that the ancestral states that we have inferred could change with additional data since the spine of the phylogenetic tree has low support. Still, the reconstruction of the character states in many ways agrees with the history of white sand, terrace and clay soils in the Western Amazon.

A majority of Protieae species have an Amazonian distri-

TABLE 5. The number of gains for each soil association under stochastic character mapping over all 40,000 Bayesian trees.

Soil type	Number of gains	Frequency
Clay	0	0.0006
	1	0.035
	2	0.203
	3	0.101
	4	0.068
	5	0.115
	6	0.091
	7	0.072
	8	0.064
	9	0.051
	10	0.052
	11	0.052
	12	0.040
	13	0.031
	14	0.017
	15	0.007
	16	0.002
	17	0.0006
	18	0.00003
Terrace	0	0.050
	1	0.147
	2	0.112
	3	0.105
	4	0.095
	5	0.075
	6	0.065
	7	0.112
	8	0.145
	9	0.065
	10	0.020
	11	0.005
	12	0.001
	13	0.001
	14	0.0001
White sand	0	0
	1	0.0001
	2	0.0003
	3	0.0006
	4	0.012
	5	0.968
	6	0.018
	7	0.0002
	8	0.00003

bution (Daly 1987). Combining molecular sequence data with dated Protieae fossils, Weeks et al. (2005) estimated that the origination of the Protieae occurred 25–54 million years ago. This range in age is large, but dates before the Miocene (23.8–5.3 million years ago), and hence before the Andean uplift. In the early Miocene, the western Amazon was receiving sediments from the east, mostly eroded rock from the Brazilian and Guianan shields (Hoorn 1993). These sandstone rocks have a largely Precambrian origin and sediments derived from them would be without question of lower fertility than the younger Cretaceous-aged rocks that would appear in the mid-Miocene (Struwe et al. 1997). Therefore, the majority of the area of the western Amazon before the Andean uplift must have been covered by white sands and other deposits similar to the brown-sand soils of intermediate fertility found today on the Brazilian and Guianan shields (Struwe et al. 1997).

White-sand soils were almost certainly more extensive in the western Amazon (and in the entire Amazon basin) than they are today (Ab'Sáber 1982; Struwe et al. 1997). If so, white-sand specialist taxa might be expected to be an earlier offshoot in this clade such as was inferred in the Gentianaceae (Struwe et al. 1997). In contrast, white sand-association is a derived trait for the Protieae (Fig. 4, Tables 4, 5). However, if loss of large areas of white-sand habitat were accompanied by increased extinction of white-sand specialists (or shifts to other habitats), the plesiomorphic state of white-sand association would be obscured in our character state reconstruction and in our phylogenetic analyses.

In the mid-Miocene the uplift of the Andes caused the northwest Amazon to be flooded with sediments of more recent origin and higher fertility. This event represented the creation of a new edaphic habitat, clay, covering an immense area from the base of the Andes to more than 500 km east (Hoorn 1993, 1994). Today, even though a substantial area of the western Amazon has been subsequently covered by less fertile terrace soil (Hoorn 1993), vast areas of relatively fertile clay soil still cover most of the lowlands within 50–200 km east of the Andes (Huston 1994).

Thus, the prevalence of clay specialists arising from sandy-soil associated ancestors in the Protieae corresponds with the great increase in area of clay soil habitat starting in the mid-Miocene. There are other species-rich genera in the Amazon that are especially diverse in the western Amazon. For example, the explosive radiation of the genus *Inga* (Fabaceae) was dated as occurring post–Andean uplift and most *Inga* species occur in the foothills of the Andes on clay soils (Richardson et al. 2001). As more phylogenetic studies are conducted on other taxonomic groups of plants that have a pan-Amazonian distribution, we can further test the hypothesis that western Amazonian species associated with clay soils are more recently derived.

Several studies on Amazonian birds, primates, and marsupials at a variety of different phylogenetic scales have found that western Amazonian species (or haplotypes) are more recent derivatives of clades that earlier had central Amazonian and Atlantic forest distributions (Silva and Oren 1996; Bates et al. 1998; Patton et al. 2001; Marks et al. 2002). While these specific studies did not investigate edaphic specialization patterns within the geographic regions, some other animals are known to be endemic to edaphic habitats in the Amazon. In the last decade there have been six new birds discovered in the Peruvian white-sand forests listed in Figure 1 (Whitney and Alvarez 1998; Alvarez and Whitney 2001, 2003; Isler et al. 2001). Molecular-based studies of these birds have yet to be conducted, and it is unknown whether these species are ancient habitat specialists from a previously more widespread habitat (like the Gentianaceae) or are more recently derived (like the Protieae may be).

Soil Association Has Repeatedly and Independently Evolved in the Protieae

If edaphic specialization were a conservative trait in the Protieae, the extreme would be one shift for each habitat specialization event, meaning that white-sand, terrace, and clay specialization had evolved only once in the group. At the other extreme of pronounced evolutionary lability, edaphic specialization would be involved in every single diversification event, and the number of changes would equal the number of species that were edaphic specialists. Our results fall in between these two expectations. On one hand, there are instances where putative sister taxa share the same soil association (Fig. 3). Phylogenetic niche conservatism, in this case association to soil type, can actually increase rates of allopatric speciation if adaptation to novel soil types is constrained and prevents the evolution of generalist species that can cross habitat boundaries (Wiens 2004). On the other hand, our phylogenetic analyses indicate that association with each of the three soil types has evolved independently multiple times (Tables 4, 5) with putative sister taxa associated with different soil types, consistent with the hypothesis that ecological speciation is driving evolutionary divergence (Fig. 3). Moreover, these results are likely only a conservative estimate of the amount of ecological speciation that has occurred in the Protieae because adding additional taxa could only increase the minimum number of habitat shifts in the clade. Overall, the data suggest that environmental heterogeneity is involved in the diversification process for many species of Protieae, especially for clay and white-sand associated species.

Evidence is accumulating that the repeated independent evolution of habitat specialist taxa within closely related groups of species appears to be a common phenomenon in plants. For example, in the extraordinarily diverse Mediterranean zones of South Africa and Australia, more than 90% of the endemic species were edaphic specialists, many of them belonging to speciose genera that include multiple edaphic specialists on different substrata (Cowling et al. 1994). Phylogenetic studies of plants endemic to serpentine soils in California have found that serpentine specialists have evolved multiple times within several phylogenetically distant genera (Pepper and Norwood 2001; Rajakaruna et al. 2003; Patterson and Givnish 2004). Several species of sympatric habitat-specialist oaks (*Quercus*) were found to not be each other's closest relatives, indicating that habitat specialization has evolved repeatedly (Cavender-Bares et al. 2004). In a phylogenetic study of 20 species of the Melastomataceae liana *Adelobotrys* from the Amazon basin, Schulman (2003) found three putative sister pairs with divergent edaphic associations. These examples, together with the results from our study point to an active role for edaphic heterogeneity in the diversification process.

Potential Mechanisms of Edaphic Specialization in the Amazonian Biota

There are two competing hypotheses that relate to the splitting of a lineage into two species with different edaphic tolerances in the Amazon Basin. The first hypothesis, the edaphic refuge hypothesis, is a modification of the vanishing refuge hypothesis (Vanzolini and Williams 1981; Moritz et al. 2000). This hypothesis contends that climatic events caused lowland forest in the Amazon to fragment and populations to become isolated from one another; (Haffer 1969; Haffer and Prance 2001). If isolated populations were confined to areas with different edaphic conditions, selection to adapt to the local soils in the refuges combined with the cessation of gene flow from other populations in different soil types could result in diversification by allopatric speciation. Subsequent wetter climates then would permit range reexpansion and result in overlapping distributions for the newly split sister taxa, with each new species confined to different soil types. Whether the Amazon basin actually experienced aridity or forest fragmentation during glacial periods is not clear and is the subject of active debate (Bush 1994; Colinvaux et al. 2000; van der Hammen and Hooghiemstra 2000; Haffer and Prance 2001).

The second hypothesis, known as the gradient hypothesis, posits that diversification can occur without allopatric isolation. If two adjacent habitats select for different traits in the two populations and hybrids of the incipient species are at a selective disadvantage, species may then diverge as a result of parapatric speciation across a sharp environmental gradient (Endler 1977, 1982). While our results are consistent with the predictions of the gradient hypothesis, phylogenies alone cannot serve as an explicit test of this idea. Instead, they should be viewed as an obligatory first step in the evaluation of the role of the gradient hypothesis in a clade of habitat specialist species. Thus, deciding which model (gradient or edaphic refuge) appears more likely to explain sister taxa with divergent soil preferences depends on whether trees can indeed undergo morphological and genetic divergence in the presence of gene flow. To date, this has not been tested.

Edaphic specialists may have arisen by mechanisms described by either the gradient hypothesis or the edaphic refuge hypothesis. In either case, we argue that speciation scenarios that ignore the contribution of edaphic heterogeneity are incomplete. The next step will be to investigate the individual, recently derived sister taxa that live in adjacent but distinct habitats (Fig. 3). These species pairs, if currently undergoing parapatric speciation, ought to exhibit morphological or reproductive divergence even in the presence of measurable gene flow (Orr and Smith 1998; Moritz et al. 2000; Levin 2004). Then, reciprocal transplant experiments of habitat-specialist trees (cf. Fine et al. 2004) can reveal which genetically based traits are required for edaphic specialization. In addition, the species of Protieae that were associated with more than one habitat could represent ecotypes that are possibly undergoing incipient speciation. Increased phylogeographic sampling, together with population genetics studies across ecotones should prove fruitful in understanding the mechanism by which soil specialization drives diversification in the Amazonian flora.

Conclusions

This study represents an initial attempt to document the prevalence of edaphic specialization and to understand the mechanisms behind the evolution of edaphic specialists in a common and diverse group of Amazonian trees. More complete surveys throughout the geographic range of these species will likely refine our understanding of edaphic specialization, and even change some of our characterizations. However, this effort is one of the most large-scale phylogenetic studies to date in any group of Amazonian trees and the only one to specifically focus on edaphic habitats. In the western Amazon, almost three-quarters of the species of Protieae we sampled are associated with only one of the three soil types we surveyed—strong evidence that edaphic heterogeneity increases the regional diversity for this clade of Amazonian trees. When examining the phylogenetic patterns of edaphic specialists, we found that soil associations have repeatedly and independently evolved and that edaphic heterogeneity has played an important role in the diversification of many groups within the Protieae. The presence of multiple putative sympatric sister taxa with divergent soil associations is consistent with both allopatric and parapatric speciation scenarios. Future work should focus on the mechanisms of how reproductive isolation may evolve in edaphic specialist plants.

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