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Comparison of Genetic Variation in Bird-Dispersed Shrubs of a Tropical Wet Forest¹

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ABSTRACT

Genetic variation was surveyed in three understory plant species in tropical wet forest at Estación Biológica La Selva, Costa Rica: *Henriettea tuberculosa* (Melastomaceae), *Psychotria officinalis* and *P. suerrensis* (Rubiaceae). These three shrub species showed moderate to high levels of genetic variation, but *Psychotria officinalis* and *P. suerrensis* had a higher proportion of polymorphic loci and greater levels of heterozygosity than did *Henriettea tuberculosa*. All three species have insect-pollinated flowers and bird-dispersed berries; the two *Psychotria* species are distylous and self-incompatible. Lower levels of genetic variation in *Henriettea tuberculosa* are hypothesized to be a result of lower outcrossing rates and local seed movement.

RESUMEN

Se estudió la variación genética de tres plantas de sotobosque en el bosque muy húmedo tropical de la Estación Biológica La Selva, Costa Rica: *Henriettea tuberculosa* (Melastomataceae), *Psychotria officinalis* y *P. suerrensis* (Rubiaceae). Estos tres arbustos mostraron grados de variación genética de moderados a altos, no obstante *Psychotria officinalis* y *P. suerrensis* tuvieron una proporción más alta de loci polimórficos y mayores niveles de heterocigosidad que *Henriettea tuberculosa*. Las tres especies tienen flores polinizadas por insectos y bayas con semillas dispersadas por aves; las dos especies de *Psychotria* son distílicas y autoincompatibles. Se hipotetiza que los niveles de variación genética en *Henriettea tuberculosa* son mas bajos como resultado de menores grados de polinización cruzada y movimiento local de semillas.

Key words: bird dispersal; electrophoretic variation; Costa Rica; genetic variation; Melastomataceae; mutualism; Rubiaceae; seed dispersal; tropical shrubs.

THE VAST MAJORITY OF SHRUBS in tropical wet forests have pollination and seed dispersal systems adapted for mutualistic interactions with animals (Howe & Smallwood 1982, Gentry 1988). Indeed, 96 percent of shrubs in a Costa Rican wet forest are pollinated by animals (Kress & Beach 1994) and 98 percent produce fleshy fruits (Opler *et al.* 1980). Thus, attraction of animals for pollination and/or seed dispersal is critically important for gene flow through both pollen and seed movement. For self-incompatible species, attraction of pollinators is essential for reproduction. Whereas, for species with self-compatible breeding systems, selfing rates may be quite high unless attraction of pollinators is highly effective. The extent to which gene flow occurs through seed dispersal may differ across plant species because seed dispersal agents differ greatly in their effectiveness (*e.g.*, Howe 1980, Loiselle 1990, Schupp 1993). Thus, genetic variation in understory

shrubs will be influenced strongly by mutualistic interactions with animals, as well as breeding systems of plants, selection, past historical population processes, and chance events (Hamrick & Godt 1989).

Because knowledge about genetic variation in tropical shrub species is based on relatively few sites and few species (Hill *et al.* 1978, Hamrick & Loveless 1986, Hamrick & Godt 1989, Loveless 1992, Pérez-Nassar *et al.* 1993), the goal of our study was to examine and compare patterns of electrophoretically detected genetic variation in several common understory shrubs in a Costa Rican tropical wet forest. Generally, genetic variation in tropical shrub species are relatively high (Hill *et al.* 1978, Hamrick & Loveless 1986, Hamrick & Godt 1989, Loveless 1992, Pérez-Nassar *et al.* 1993) although one study reports very low amounts (Heywood & Fleming 1986). The species used in this study were all fleshy-fruited bird-dispersed shrubs with only slightly different life history characteristics. They were relatively common elements of the forest understory and possessed a range of pollinators, avian

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seed dispersal agents, and flower and fruit phenologies. Information on these species will illustrate how patterns of genetic variation in this tropical wet forest site compare with other tropical locations, as well as provide insight for comparison among the selected species. The five study species included three species of Melastomataceae (*Clidemia densiflora*, *Miconia simplex*, and *Henriettea tuberculosa*) and two species of Rubiaceae (*Psychotria officinalis*, *P. suerrensii*). This paper focuses on the latter three species because they were the ones which exhibited interpretable enzyme activity.

METHODS

STUDY SITE AND SPECIES.—This study was conducted in undisturbed forest at Estación Biológica La Selva, a field station operated by the Organization for Tropical Studies (OTS) in northeast Costa Rica. La Selva encompasses 1536 ha and is adjacent to the much larger Parque Nacional Braulio Carrillo (ca. 44,000 ha). La Selva receives approximately 4000 mm of rain annually (McDade & Hartshorn 1994, Sanford *et al.* 1994). The main dry season lasts from January or February to March or April with a shorter, less pronounced dry season in September or October. The forest at this site is classified as tropical wet forest following the Holdridge (1967) life-zone classification (Hartshorn & Peralta 1988). A complete description of this site is available in McDade *et al.* (1994 and refs. therein).

The three study species in the Melastomataceae are: *Henriettea tuberculosa* (Donn. Sm.) L. O. Wms., *Clidemia densiflora* (Standl.) Gleason, and *Miconia simplex* Tr. The two species of Rubiaceae are: *Psychotria officinalis* (Aubl.) Sandw., and *P. suerrensii* Donn. Sm. These species are relatively common on the long-term plot studied by B. A. Loiselle and J. G. Blake (*e.g.*, Blake & Loiselle 1991; Loiselle & Blake 1991, 1993) and considerable information already has been gathered on aspects of their seed dispersal ecology.

Individuals of *H. tuberculosa* are the largest among the five species, reaching heights of 6–8 m and diameters of 8–10 cm at breast height. Although this understory tree can be found from ridges to valley bottoms, it appears to be most common on lower slopes and bottomland/swale areas (B. Loiselle, pers. obs.). Small white hermaphroditic flowers are visited by small bees, but the breeding system is unknown (Renner 1989, Kress & Beach 1994). Individuals often produce from 6000–10,000 orange berries, which are produced seasonally along twigs and branches (B. Loiselle, pers.

obs.). Understory birds (especially *Euphonia gouldi*, *Corapipo leucorhoa*, *Chlorothraupis carmioli*, and *Pipra mentalis*) remove the fruits and disperse the seeds (Loiselle & Blake 1990, 1993). Fruits generally contain from 75 to 175 seeds (B. Loiselle, pers. obs.).

Clidemia densiflora is an understory shrub that reaches 1.5–2 m height. Hermaphroditic flowers are pollinated by small bees (Renner 1986, pers. comm., as cited in Kress & Beach 1994) and produced in clusters along the stem and twigs. Orange berries contain many small seeds (generally ranging from 100–250 seeds/fruit, B. Loiselle, pers. obs.) and are dispersed by birds, especially *Chlorothraupis carmioli*, *Pipra mentalis*, and *Corapipo leucorhoa* (Loiselle & Blake 1990, 1993). The other melastome species is *Miconia simplex*, an understory treelet reaching 3–4 m in height. Small, pink hermaphroditic flowers are produced in terminal inflorescences; pink immature berries turn to purple-black at maturity and generally contain from 50 to 150 seeds (B. Loiselle, pers. obs.). Berries are bird-dispersed and are most often found in the diets of *Pipra mentalis* and *Mionectes oleagineus*.

The two Rubiaceae species are often found side by side in La Selva forests. Although these plants occur in many microhabitats, *Psychotria officinalis* is relatively more common in upland areas, whereas *P. suerrensii* appears to be relatively more widespread. Individuals of the former tend to be larger and can reach heights of 5–6 m and 5 cm DBH, whereas *P. suerrensii* rarely exceed 2–2.5 m height. Both species are distylous and self-incompatible (Bawa & Beach 1983). *Psychotria officinalis* is pollinated by small bees and butterflies, while *P. suerrensii* appears to be pollinated primarily by butterflies and occasionally *Phaethornis longuemareus* hummingbirds (Bawa & Beach 1983, Kress & Beach 1994; J. Stone, pers. comm.). Berries of both species are dispersed primarily by understory birds (especially manakins) and contain two seeds per fruit (although only one seed may develop fully).

ELECTROPHORETIC STUDIES.—To conduct the enzyme trial runs, leaves from the five understory shrub species were collected on several research trips (March 1991, January 1992, June 1992, and July 1993) using different techniques for leaf preparation. During the first two research trips, 2–3 leaves were collected from approximately 50 individuals. Leaves were kept on ice in a cooler and then either vacuum-dried immediately (for either 24 or 48 hours) or placed in an ultra-cold freezer (–70°C) until vacuum-drying. Following drying, they were stored in

sealed plastic bags. Leaves were then transferred to an ultra-cold freezer at UM-St. Louis and later extracted. During June 1992 leaves were collected from adult plants in a similar manner but kept on ice until return to St. Louis (3 days) where they were immediately placed in an ultra-cold freezer until extraction. During the July 1993 collecting trip, leaves were kept on ice in the field and transferred to a liquid nitrogen tank on the same day. Upon return to St. Louis, leaves were transferred to an ultra-cold freezer. Adequate electrophoretic results were obtained from both vacuum-dried (48 hours only) and leaves kept on ice, but best results were obtained using leaves stored in the liquid nitrogen tank and those results are reported here.

Allozyme analyses generally followed methods described by Soltis *et al.* (1983) and Hamrick and Loveless (1986). We made extracts from leaf material of all five species using two phosphate-polyvinylpyrrolidone extraction buffers (Mitton *et al.* 1979, Wendel & Parks 1982). Leaves from an individual plant were divided into two parts and ground in separate mortar and pestles using liquid nitrogen. Each of the paired samples of powdered leaf material was then extracted with one of the extraction buffers. We used filter paper cut into 0.6 × 0.4 cm pieces as wicks to absorb resultant crude extracts; approximately 12 wicks were taken from each individual plant and stored in an ultra-cold freezer until gels were ready to be run.

Following a protocol developed by J. Hamrick (pers. comm.), we performed enzyme trial runs on all five species with wicks taken from each extraction buffer. These trial runs were designed to determine which buffer systems and which enzymes produce the clearest banding patterns. Electrophoresis was conducted on 10 percent potato starch gels (Sigma S-4501). A total of six buffer systems were used and 25 enzymes were stained in these runs. Recipes for gel buffer systems (4, 7, 10, 11) are available in Soltis *et al.* (1983); buffer system 34 reported in Table 1 is equivalent to system 6 in Soltis *et al.* (1983) and buffer system 8⁻ is a modification of Soltis *et al.* (1983) system 8 (J. Hamrick, pers. comm.). To determine which enzymes showed interpretable banding patterns, we graded the activity of each using a rating scale that ranged from A (excellent) to F (no activity); only scores of B⁻ or better were used in later gel runs to examine genetic variation at putative loci. Starch gels were then repeated for approximately 45–60 individuals for three of the five plant species using the enzymes and buffer systems empirically determined to give

the most reliable results (see Table 1 for summary of successful systems).

DATA ANALYSIS.—For comparison of genetic diversity, we used leaves from the three species that were collected over an approximate 1 ha area in undisturbed forest on residual soils derived from lava flows (Sollins *et al.* 1994). For each species we sampled individuals spaced 2–5 m apart within a contiguous area.

We used BIOSYS-1 (Swofford & Selander 1981) to compute allelic frequencies and measures of genetic variability, as well as to test for deviation from Hardy-Weinberg expectations using Chi-square. Genetic variability measures reported here include the proportion of polymorphic loci (*P*), mean heterozygosity per locus based on Hardy-Weinberg expectations (H_e) and direct count (H_o), and mean number of alleles per polymorphic locus (*A*). *P* was determined by dividing the number of polymorphic loci by the total number of loci scored. *A* was calculated by summing observed alleles over all polymorphic loci and dividing by total number of loci. Direct count or observed heterozygosity is the average heterozygosity of individuals within populations (Nei 1975). H_e is calculated from observed allelic frequencies.

RESULTS

TRIAL RUNS.—We tested 25 enzymes for isozyme variation using two extraction buffers and a total of six gel buffer systems for each shrub species; 17 of 25 enzymes tested showed adequate activity for further analysis (Table 1). Two species, *M. simplex* and *C. densiflora*, showed little or no activity on starch gels for all enzymes and buffer systems tested and, thus, no electrophoretic results are reported (Table 1). Trials were run three times (once in the laboratory of J. Hamrick) to insure that results were not due to leaf collection and storage techniques or laboratory methods.

Trial runs using tissue from *H. tuberculosa* revealed adequate activity in 10 enzymes and overall better electrophoretic performance from leaves extracted using the extraction buffer described in Mitton *et al.* (1979) (Table 1); 9 enzymes were readily interpretable. For the two *Psychotria* species, leaves extracted from extraction buffer described in Wendel and Parks (1982) produced overall better electrophoretic results, although results were also interpretable from the Mitton *et al.* (1979) extraction buffer. For each *Psychotria* species, 11 enzymes on

TABLE 1. Gel and electrode buffer systems, polymorphism levels, and quaternary structure for enzymes showing activity in electrophoretic studies of three understory plant species. For further details on gel and electrode buffers see methods section. Extraction buffers are described in the methods. Extraction buffers used here were from Mistron et al. (1979) for *Henriettea tuberculosa* and from Wendel and Parks (1982) for the two *Psychotria* species. For polymorphic loci (p), quaternary structure is indicated; m is monomer, and d is dimer. Enzymes ACO, ACP, ADH, ALD, F-1,6, GAL, GDH, and MPI showed no activity on any buffer systems and are not included in this table; additional gel buffer systems used were 7 and 10 (see Soltis et al. 1983). Results from identical electrophoretic trials on *Clidemia densiflora* and *Miconia simplex* are not summarized as no enzymes ever showed adequate activity. Names, abbreviations, and enzyme commission numbers (I.U.B. 1984) follow the table.

Enzyme ^b	<i>H. tuberculosa</i>			<i>P. suerrensii</i>			<i>P. officinalis</i>		
	Buffer system	Monomorphic/polymorphic	Quaternary structure	Buffer system	Monomorphic/polymorphic	Quaternary structure	Buffer system	Monomorphic/polymorphic	Quaternary structure
AAT-1				8-	p	d			
AAT-2				8-	p	d	8-	m	
AK-2							11	m	
CE-1				34	m		34	p	m
CE-2				34	p				
DIA-1	34	m		34	m	m			
DIA-2	34	m							
FE-1	8-	m							
FE-2	8-	m		8-	m		34	p	m
GDH-1				34	m		8-	p	m
G6PD-1				11	p	d	11	p	d
G6PD-2				11	p	d	11	p	d
GPI-1	34	m		34	m		11	m	
GPI-2				34	p ^a		11	p	d
IDH-1				11	p	d	11	p	d
LAP-1	34	m					8-	p	m
LAP-2							8-	p	m
MDH-1	4	m		11	p ^a				
ME	8-	p ^a							
MNR-1	34	m		8-	p	m	34	p ^a	
MNR-2				8-	p	m			
PER-1	34	p	m				11	m	
PGM-1				11	m				
PGM-2				11	m				
SKDH-1	4	p	m	8-	m		34	m	
TPI-1	8-	p	d	8-	m		34	m	
TPI-2	8-	m		8-	m		34	m ^a	
TPI-3				8-	p	d			

^a Polymorphic loci, but could not be interpreted consistently.

^b Standard enzyme codes: aspartate amino transferase, EC 2.6.1.1.-AAT; aconitase, EC 4.2.1.3.-ACO; acid phosphatase, EC 3.1.3.2.-ACP; alcohol dehydrogenase, EC 1.1.1.1.-ADH; adenylate kinase, EC 2.7.4.3.-AK; aldolase, EC 4.1.2.13.-ALD; colorimetric esterase, EC 3.1.1.1.-CE; diaphorase, EC 1.6.4.3.-DIA; fluorescent esterase, EC 3.1.1.-FE; fructose-1,6-diphosphatase, F-1,6; 13-galactosidase, EC 3.2.1.23.-GAL; guanine aminohydrolase, EC 3.5.4.3.-GDH; glucose-6-phosphate dehydrogenase, EC 1.1.1.49.-G6PD; glucosephosphate isomerase, EC 5.3.1.9.-GPI; isocitrate dehydrogenase, EC 1.1.1.42.-IDH; leucine aminopeptidase, EC 3.4.11.1.-LAP; malate dehydrogenase, EC 1.1.1.37.-MDH; malic enzyme, EC 1.1.1.40.-ME; mannose reductase, MNR; mannosephosphate isomerase, EC 5.3.1.8.-MPI; peroxidase, EC 1.1.1.17.-PER; phosphoglucomutase, EC 2.7.5.1.-PGM; shikimate dehydrogenase, EC 1.1.1.25.-SKDH; triosephosphate isomerase, EC 5.3.1.1.-TPI.

three buffer systems showed easily interpretable results (Table 1).

ALLOZYME VARIATION.—Little allozyme variation was detected among 60 *H. tuberculosa* individuals sampled (Table 2). Only 3 of 12 putative loci were polymorphic, resulting in relatively low values for mean heterozygosity (Table 3). In contrast, the two *Psychotria* species had greater allozyme variation (Table 2). In *P. suerrensis*, six of 11 enzymes and nine of 18 putative loci showed some degree of variation. Although in three of those nine polymorphic loci (MNR-1, TPI-3, 6-GPD-1) the second allele was rare in the population (*i.e.*, found in <5% of individuals) (Table 2). In *P. officinalis*, 7 of 11 enzymes and 9 of 14 putative loci showed genetic variation. Of the three species, *P. officinalis* showed the greatest amount of genetic variation in all three genetic variability measures (Table 3).

For polymorphic loci of each plant species, we tested for deviation in genotype frequencies from that expected by Hardy-Weinberg (1:2:1 ratios) using Chi-square tests. No such deviations were found for polymorphic loci of *H. tuberculosa* ($\chi^2 < 3.9$, $P > 0.25$ in all cases). For *P. suerrensis*, however, 2 of 9 loci had significantly different genotypic frequencies than predicted by Hardy-Weinberg. MNR-1 had fewer heterozygotes than expected ($\chi^2 = 12.5$, $df = 1$, $P < 0.001$), whereas AAT-1 had more heterozygotes than expected ($\chi^2 = 18.5$, $df = 1$, $P < 0.001$). Similarly, 2 of 9 loci showed significant deviations from expected Hardy-Weinberg frequencies in *P. officinalis*; FE-1 had no observed heterozygotes ($\chi^2 = 81.0$, $df = 1$, $P < 0.001$), whereas LAP-1 had more heterozygotes than expected ($\chi^2 = 5.5$, $df = 1$, $P < 0.02$). In all cases, we rechecked the gels to ensure that deviations were not due to errors in misinterpretation of banding patterns.

DISCUSSION

In our trial runs, several attempts with two extraction buffers failed to reveal any genetic markers for *C. densiflora* and *M. simplex*. Where there is a need for more information on patterns of genetic variation in shrubs of tropical wet forests, not all species are equally amenable to electrophoretic analysis. Presence of secondary compounds within leaves of these two species may be responsible for poor results. However, other families (*e.g.*, Solanaceae, Fagaceae) which are known to contain many secondary compounds have shown activity in our lab. Perhaps, poor results are simply due to very rapid degradation

TABLE 2. Allozyme frequencies and single locus heterozygosity (direct count) estimates for three species of understory shrubs at Estación Biológica La Selva. N is the number of individuals sampled for each locus. For each locus and plant species, allele 1 represents the most common allele, but not the same allele across species.

Locus	Allele	<i>Henriettea tuberculosa</i>	<i>Psychotria suerrensis</i>	<i>Psychotria officinalis</i>
AAT-1	1 (N)		0.536 (42)	
	2		0.464	
AAT-2	1 (N)		0.721 (43)	1.000 (44)
	2		0.279	
AK-2	1 (N)			1.000 (43)
CE-1	1 (N)			0.042 (24)
	2 (N)		1.000 (48)	0.875
	3			0.083
CE-2	1 (N)		0.919 (43)	
	2		0.012	
	3		0.070	
DIA-1	1 (N)	1.000 (60)	1.000 (48)	
DIA-2	1 (N)	1.000 (60)		
FE-1	1 (N)			0.024 (41)
	2 (N)	1.000 (60)		0.976
FE-2	1 (N)	1.000 (60)	1.000 (48)	
GDH-1	1 (N)			0.098 (41)
	2			0.378
	3 (N)		1.000 (48)	0.524
G6PD-1	1 (N)			0.586 (35)
	2 (N)		0.967 (46)	0.414
	3		0.033	
G6PD-2	1 (N)		0.022 (45)	0.628 (43)
	2		0.789	0.372
	3		0.189	
GPI-1	1 (N)	1.000 (60)	1.000 (45)	1.000 (44)
GPI-2	1 (N)			0.349 (43)
	2			0.651
IDH-1	1 (N)		0.070 (43)	0.095 (21)
	2		0.767	0.905
	3		0.163	
LAP-1	1 (N)			0.360 (43)
	2 (N)	1.000 (60)		0.640
LAP-2	1 (N)			0.066 (38)
	2			0.934
MDH-1	1 (N)	1.000 (60)		
MNR-1	1 (N)	1.000 (60)	0.951 (41)	
	2		0.049	
MNR-2	1 (N)		0.845 (42)	
	2		0.155	
PER-1	1 (N)	0.110 (59)		
	2	0.890		
PGM-1	1 (N)		1.000 (48)	1.000 (44)
PGM-2	1 (N)		1.000 (48)	
SKDH-1	1 (N)	0.085 (59)		
	2	0.169		
	3	0.746		
TPI-1	1 (N)	0.920 (56)	1.000 (48)	1.000 (43)
	2	0.080		
TPI-2	1 (N)	1.000 (60)	1.000 (48)	
TPI-3	1 (N)		0.010 (48)	
	2		0.990	

TABLE 3. Summary of genetic variability measures for preliminary isozyme analysis of three understory shrubs at Estación Biológica La Selva: mean direct count heterozygosity per locus, mean expected heterozygosity based on Hardy-Weinberg expectations (HW), mean number of alleles per polymorphic locus, and percent polymorphic loci (criteria for common allele is 0.95). Standard error values are provided in parentheses.

Variability measures	<i>Henriettea tuberculosa</i>	<i>Psychotria suerrensis</i>	<i>Psychotria officinalis</i>
Mean heterozygosity:			
direct count (SE)	0.073 (.044)	0.141 (.054)	0.188 (.052)
HW (SE)	0.063 (.037)	0.123 (.041)	0.215 (.060)
Mean number of alleles per polymorphic locus (SE)	1.33 (.19)	1.67 (.18)	1.79 (.19)
Percent polymorphic loci	25.0	33.3	57.1

of plant enzymes. Regardless, future work with these species will require special efforts to discover the techniques necessary to obtain enzyme activity.

Of the three species in which we found adequate enzyme activity, the two *Psychotria* species showed greater heterozygosity, allelic diversity, and polymorphism than did *H. tuberculosa*. Levels of genetic variation for the two *Psychotria* species generally matched those for other *Psychotria* reported elsewhere. Observed heterozygosity values and percent polymorphic loci reported for *Psychotria faxlucens* ($H = 0.198$, $P = 40\%$; Pérez-Nasser *et al.* 1993) and *Psychotria horizontalis* ($H = 0.133$, $P = 49.5\%$; Hamrick & Loveless 1989) were similar to the two *Psychotria* studied here. Mean number of alleles per polymorphic loci, however, was considerably higher for *P. faxlucens* ($A = 2.50$, Pérez-Nasser *et al.* 1993). In general, levels of genetic variation found for the three La Selva species matched those reported by Hamrick and Loveless (1986) for tropical shrub species on Barro Colorado Island in Panama. Mean heterozygosity of BCI understory species ranged from 0.046 (*Rinorea sylvatica*: Violaceae) to 0.216 (*Soracea affinis*: Moraceae); percent polymorphic loci ranged from 15 percent to 53 percent. One species, *Fareamea occidentalis* (Rubiaceae), showed no genetic variation in 14 loci (Hamrick & Loveless 1986). Tropical *Piper* species from Costa Rican dry forest examined by Heywood and Fleming (1986) showed low levels of genetic diversity with from 0 to 9.5 percent polymorphic loci and mean heterozygosity levels not exceeding 0.033. In relative terms, the two *Psychotria* species exceeded the mean values of P and H reported by Loveless (1992) for tropical shrub species; *H. tuberculosa* was close to her reported mean values. Thus, genetic variation levels in *H. tuberculosa* appear typical for tropical shrubs, while the two *Psychotria* species are at the upper end.

Several explanations may account for the rela-

tively high levels of genetic variation in *Psychotria* species compared to *H. tuberculosa* and other tropical shrub species. The two *Psychotria* species are self-incompatible which presumably enforces high outcrossing rates and promotes high genetic variation (Loveless & Hamrick 1984, Brown 1989). Although the breeding system for *H. tuberculosa* is not known, its lower levels of genetic variation might indicate that this species has lower outcrossing rates. Differences in genetic variation among these shrub species, however, may also be due to the seed dispersal dynamics. For example, the large fruit crops of *H. tuberculosa* result in birds remaining in the tree or nearby for several minutes (B. Loiselle, pers. obs.). As defecation rates for birds are rapid (Levey 1986, 1987; Martínez del Rio & Restrepo 1993), many seeds are likely defecated near the maternal plant. Moreover, hundreds and even thousands of *H. tuberculosa* seeds often are deposited in the same fecal clump (B. Loiselle, pers. obs.). Such dispersal events would tend to restrict gene flow. On the other hand, the higher levels of variation in *Psychotria* could be influenced by more extensive gene flow through seed dispersal. For example, female manakins, a major dispersal agent for *Psychotria* species, travel over long distances in the forest understory to visit widely dispersed male lek sites. Of course, the complex nature of the dynamics of pollination and seed dispersal require that detailed ecological studies be completed before we will be able to precisely understand why some shrub species show greater genetic variation than others.

In conclusion, the relatively high levels of genetic variation for *P. officinalis* and *P. suerrensis* compared to other tropical shrub species are consistent with predictions for self-incompatible, animal pollinated and dispersed shrub species. The relatively low level of genetic variation for *H. tuberculosa*, could be due to lower outcrossing rates, to restricted seed dispersal or some combination of the

above. Our findings demonstrate that considerable variation occurs even among plant species with similar life history characteristics and seed dispersal agents.

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