

Reproductive ecology of 21 coexisting *Psychotria* species (Rubiaceae): when is heterostyly lost?

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Heterostyly is a genetic polymorphism in which plant populations are composed of two or more morphs that differ in stigma and anther heights. The polymorphism promotes intermorph pollen transfer, thus outcrossing. Heterostyly has been reported in 28 angiosperm families and is frequently lost in heterostylous lineages. To assess ecological factors related to shifts from heterostyly to monomorphy, we examined the reproductive ecology of heterostylous tropical shrubs of *Psychotria*. Among 21 species at Barro Colorado Island and the nearby Parque Nacional Soberania, Panama, 14 species were heterostylous while seven were monomorphic. A molecular phylogeny and the existence elsewhere of heterostylous populations indicated that the breakdown of heterostyly had occurred independently. Heterostylous and monomorphic species were visited by the same bee species, although visit frequencies were lower in monomorphic species. Monomorphic species had significantly lower population density and greater fruit set than did heterostylous species. Autonomous autogamy made a large contribution to fruit set in monomorphic species and was only rarely observed in heterostylous species. The results indicate monomorphic and heterostylous species produce more seeds through selfing and outcrossing, respectively. The limitation of outcrossing as a result of low population density may be related to the breakdown of heterostyly and the evolution of selfing. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, 93, 125–134.

ADDITIONAL KEYWORDS: Barro Colorado Island – breeding system evolution – Panama – pollination – rarity – self-fertilization – tropical forest.

INTRODUCTION

The amazingly diverse reproductive systems of flowering plants have fascinated biologists since Charles Darwin (Darwin, 1877; Barrett, 2002a). Some plants achieve sexual reproduction only through selfing. Selfing solves the problem posed by mate limitation, with the added advantage that twice as many genes are transferred to their offspring (Fisher, 1941), but with the potential cost of inbreeding depression and loss of genetic diversity among their offspring compared with outcrossed offspring. Other plants produce seeds partly or exclusively through outcrossing. Reproductive systems that achieve outcrossing without sexual interference between male and female functions within a hermaphrodite flower or individual

(Barrett, 2002b) have evolved repeatedly and include self-incompatibility, spatial (herkogamy) and temporal (dichogamy) separation of male and female organs within a flower and floral polymorphisms. These reproductive systems may be lost again in some descendants with concomitant decreases in outcrossing rates. Evolutionary changes in reproductive systems, and thus outcrossing rates and plant characteristics, and environmental factors associated with these changes have been some of the central problems of evolutionary ecology for the last three decades (e.g. Jain, 1976; Schoen, Morgan & Bataillon, 1996; Barrett, 2003).

The breakdown of floral polymorphisms offers one of the best systems for studies of evolutionary changes in reproductive systems because the direction of evolutionary change is usually unambiguous (evolution of polymorphism is less likely than its loss), genetic modifications are often simply inherited, and

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the change is readily detected in the field as changes in floral morphology (Baker, 1959; Barrett, 1992). The breakdown is considered to be associated with decreases in outcrossing, possibly as a result of long-distance colonization (e.g. Barrett, 1985) or of decreases in pollinator availability (e.g. Washitani *et al.*, 1994; Pauw, 2005), population size or population density (e.g. Ganders, 1975). Among floral polymorphisms, heterostyly is most frequently studied (Ganders, 1979; Barrett, 1992; Barrett, Jesson & Baker, 2000). Heterostylous populations are composed of two (distyly) or three (tristyly) morphs that differ reciprocally in the heights of stigmas and anthers. The reciprocal arrangement of stigmas and anthers promotes intermorph pollen transfer (e.g. Stone & Thomson, 1994; Ree, 1997). The style-stamen polymorphism is usually accompanied by a sporophytically controlled, diallelic self-compatibility system that prevents self- and intramorph fertilizations (Darwin, 1877; Ganders, 1979; Barrett, 1992). Heterostyly has been reported in 28 angiosperm families (Barrett *et al.*, 2000) and has evolved independently at least 23 times (Lloyd & Webb, 1992). The family Rubiaceae probably include more heterostylous species than do all other families combined (Ganders, 1979).

Psychotria, Rubiaceae, is one of the largest genera of flowering plants. The genus is nearly ubiquitous in tropical forest understories, and most species are small trees and shrubs. Many *Psychotria* species are heterostylous, and heterostyly has been lost in other congeners (Burck, 1884; Vuilleumier, 1967; Hamilton, 1990; Orians, 1997). As more than 20 species may coexist in a tropical forest, the group provides a unique opportunity to examine correlations between reproductive systems and other ecological factors among sympatric species. We assess correlations between floral morphology, breeding system and other ecological traits for 21 species of *Psychotria* from central Panama.

We ask whether monomorphic flowers have mechanisms to promote self-fertilization, and assess circumstances possibly related to loss of heterostyly. These circumstances include rarity, different types of pollinators, and association with ephemeral open habitats.

MATERIAL AND METHODS

STUDY SITE AND PLANTS

This study was carried out at Barro Colorado Island (BCI; 9°9'N, 79°51'W) and the contiguous Parque Nacional Soberania (PNS; 9°10'N, 79°7'W) in Central Panama in 1999 and 2000. Both sites support seasonal rainforests with a mature canopy height of 35 m. The PNS contains relatively large permanent

Table 1. List of *Psychotria* species studied at Barro Colorado Island (BCI) and the nearby Parque Nacional Soberania (PNS) with sexual systems and floral morph and species code

Sexual system	Species name	Species code
Heterostylous	<i>P. acuminata</i> Benth.	acu
	<i>P. capitata</i> Ruiz & Pav.	cap
	<i>P. chagrensis</i> Standl.	cha
	<i>P. deflexa</i> DC.	def
	<i>P. emetica</i> Vell.	eme
	<i>P. grandis</i> Sw.	gra
	<i>P. horizontalis</i> Sw.	hor
	<i>P. ipecacuanhai</i> (Brot.) Stokes	ipe
	<i>P. limonensis</i> K. Krause	lim
	<i>P. marginata</i> Sw.	mar
	<i>P. pittieri</i> Standl.	pit
	<i>P. poeppigiana</i> Müll.Arg.	poe
	<i>P. psychotriifolia</i> (Seem.) Standl.	psy
	<i>P. pubescens</i> Sw.	pub
	Monomorphic	<i>P. brachiata</i> Sw.
<i>P. brachybotria</i> Müll. Arg.		brb
<i>P. graciliflora</i> Benth.		grc
<i>P. hoffmannseggiana</i> Müll. Arg.		hof
<i>P. micrantha</i> H.B.K.		mic
<i>P. racemosa</i> (Aubl.) Raesushel		rac
<i>P. tenuifolia</i> Sw.		10

gaps associated with streams, and a four-wheel-drive road. Mean monthly temperatures are 27 °C in April and 26 °C otherwise. Annual rainfall averages 2600 mm, most of which occurs in a single rainy season from April to December. Detailed descriptions of BCI can be found in Croat (1978).

We studied 21 *Psychotria* species at BCI and PNS (Table 1). Recently Nepokroeff, Bremer & Systema (1999) proposed the division of *Psychotria* into three genera, but we follow the nomenclature of Burger & Taylor (1993) because the new genera have not been named. Croat (1978) recorded 23 *Psychotria* species from BCI. Three of these species have not been encountered on the island in recent years (*P. carthagenesis*, *P. guapilensis* and *P. uliginosa*), and he overlooked one species (*P. graciliflora*). Two additional species, *P. erecta* and *P. uliginosa*, have been observed in the PNS. Most species are shrubs, but subshrubs (*P. emetica*, *P. ipecacuanha* and *P. tenuifolia*) and small trees (*P. grandis*) are included. They

flower at the beginning of the rainy season from May to July, with the exception of *P. marginata*, which flowers in November and December. Flowers are tubular and up to 2 cm long with five corolla lobes, a style and five anthers. Inflorescences of *P. poeppigiana* are unique in having large red bracts, while other species have green bracts of variable size. Ovaries are two locular, with the exception of *P. racemosa* with five-locular ovaries. All locules potentially develop into seeds. The fleshy fruits mature from October to January. Resident manakins and migratory thrushes disperse the seeds (Poulin *et al.*, 1999). All species flowered and fruited both in 1999 and 2000 at similar intensity, except *P. tenuifolia*, which flowered only in 2000.

SEXUAL SYSTEMS

Hamilton (1990) recognized four states of floral morphology in *Psychotria*. Heterostylous populations include individuals with pin (long-styled) flowers only and individuals with thrum (short-styled) flowers only. Pin- and thrum-monomorphic populations include only individuals with pin flowers or only individuals with thrum flowers, respectively. And monomorphic homostylous populations include individuals with stigma and anthers placed at the same height in a flower.

The state of morphology of the 21 *Psychotria* species were identified based on field surveys. We judged a population to be monomorphic when every individual examined had flowers of the same morphology. To avoid double-counting a vegetatively reproduced individual, we did not count more than one individual within a 3-m distance for species with active vegetational growth. Assuming that the two morphs occur at equal frequency, which is typical of heterostylous populations, this judgement is statistically reliable when sample size is six or more (binomial test, $P < 0.031$). In central Panama, several species have only pin or thrum flowers, but are reported to be dimorphic elsewhere (Hamilton, 1990; Orians, 1997).

We used a strict consensus tree based on the internal transcribed spacer region (ITS) of nuclear ribosomal DNA to evaluate the possible independent loss of heterostyly in species with a single morph throughout their distributions. The strict consensus tree included 17 of the 21 study species (Nepokroeff *et al.*, 1999). Two character states, monomorphic or heterostylous, were mapped onto the phylogeny using MacClade 4.0 (Maddison & Maddison, 2000). We assumed transitions from heterostyly to monomorphy require only one step, while transitions in the opposite direction require more steps, as the gain of a complex trait such as heterostyly is likely

to be more difficult than its loss (Kohn *et al.*, 1996; Schoen *et al.*, 1997). More steps for the gain of heterostyly did not change the results. In the case of species that are monomorphic on BCI but heterostylous elsewhere, we assumed that the two types of populations are monophyletic. The two trichotomies were resolved in all possible ways, but the outcome of the analysis was not affected by how the trichotomies were resolved.

FRUIT SET OF OPEN AND BAGGED FLOWERS

Fruit set was surveyed for 14 BCI populations in 1999 and/or 2000. Fruit set was surveyed in both years for *P. pittieri*, *P. hoffmannseggiana* and *P. brachiata*; results were similar between years; and only results for 1999 are shown. All flowers were counted on tagged inflorescences of 8–14 individuals of each morph of each species (except *P. brachiata* with just two known flowering individuals). Sample sizes are given in Appendix S1. Flowers could not be counted without damaging the inflorescences of *P. chagrensis* because flower buds and ovaries are completely enclosed in a bract. Therefore, we destructively sampled 26 and 37 inflorescences from pin and thrum individuals, respectively, to estimate the average numbers of flowers on an inflorescence, and enumerated all inflorescences to estimate the total number of flowers per individual. About 40 days after peak flowering, the remaining ovaries (immature fruits) were counted. Most immature fruit present 40 days after peak flowering developed into mature fruit, and fruit set 40 days after flowering peak and fruit set at dispersal were strongly correlated (S. Sakai, unpublished data). For the five heterostylous species, intermorph differences in fruit set were evaluated using two-tailed *t*-tests and the Dunn–Sidak method to adjust significant levels for multiple comparisons (Sokal & Rohlf, 2001).

Relative fruit set of bagged flowers was examined in five heterostylous and five monomorphic species. Inflorescences of 1–5 individuals were enclosed in a mesh bag before flowering to exclude flower visitors, and the fruit set of enclosed flowers was investigated 40 days after peak flowering (for sample size see Appendix S2). We calculated relative fruit set of bagged flowers as fruit set of bagged flowers divided by fruit set of open flowers of the same individual. As no difference in fruit set was detected between morphs (see Results and Discussion), the fruit set of pin and thrum morphs was averaged in heterostylous species. The relative fruit set of bagged flowers for heterostylous and monomorphic species was compared using a two-tailed Wilcoxon two-sample test. All statistical tests were conducted using Statistical Analysis Software (SAS, 1988).

POLLINATORS

Only insects visited most *Psychotria* flowers. The only vertebrate visitors were hummingbirds to *P. poeppigiana*. Insect visitors were collected for 137 h in total at BCI and PNS from 07.00 to 15.30 h. Visitor faunas in a plant species did not change significantly during the day. Visitors with pollen load were judged as potentially contributing to pollination. In addition, flowers of *P. poeppigiana* visited by hummingbirds were observed from a distance > 5 m for 7 h. Hummingbirds touching both stigma and anther were judged to be potentially effective pollinators. We evaluated whether visit frequencies of pollinators (visits per hour) were different between heterostylous and monomorphic species using a two-tailed Wilcoxon two-sample test. Visitors were categorized as hummingbirds, medium-sized bees (head width > 3 mm), small bees (head width < 3 mm), wasps, or others (Diptera and Lepidoptera) (Appendix S4). Fourteen small-bee species accounted for 84% of all insects collected. Flower visitors of 16 *Psychotria* species were dominated by small bees (see Results). We calculated the Kendal coefficient of concordance to test the null hypothesis that the proportion of the 14 small bee flower visitors are dissimilar across the 16 heterostylous and monomorphic *Psychotria* species. This test leads to rejection of the hypothesis if the different bee species have similar relative counts for the 16 *Psychotria* species.

PSYCHOTRIA ABUNDANCE

All *Psychotria* stems were identified for 297 plots of 28 m² each, including 132 circular plots (3 m radius) on BCI and 165 rectangular plots (2 × 14 m) in the PNS. Plot size, shape and location insured that single habitats were included. Plots were randomly located in two habitats on BCI and four habitats in PNS. BCI habitats differed in moisture availability. 'Dry' plots (60 plots) were located 20 m south of permanent 100-m trail markers along a 6-km transect. 'Moist' plots (72) were located along six streams, near two perennial springs, and in a seasonal swamp, where the water table reaches the surface in the late wet season. PNS habitats differed in both moisture and light availability. 'Dry, edge' plots (40) were located at 100-m intervals along a 6-km transect formed by the permanent edge created by the Pipeline Road. This four-wheel-drive road was constructed in 1946 and cuts through otherwise continuous forest. 'Moist, edge' plots (43) were located on low terraces along the Quebrada Juan Grande and the Rios Agua Salud, Frijoles, Limbo and Mendoza. Understory plots were located at least 50 m from the nearest edge. 'Dry, understory' plots (58) were located on flat terrain or on shallow ridges. 'Moist, understory' plots (24) were

located in depressions and along small streams. The occurrence probability of each species was calculated as the proportion of the plots occupied for each of the six habitats, and the average and maximum values among the six were used as average and maximum occurrence probabilities, respectively. In addition, we quantified the association with edge habitats using the average occurrence probability for the two edge habitats standardized by the average occurrence probability over all six habitats. Two-tailed Wilcoxon two-sample tests were performed to evaluate if monomorphic species are different from heterostylous ones in occurrence probabilities or habitats.

RESULTS

SEXUAL SYSTEMS

Both pin and thrum morphs were found in 14 of the 21 *Psychotria* species (Table 1). Two species, *P. brachiata* and *P. racemosa*, were monomorphic homostylous. Four species, *P. graciliflora*, *P. hoffmannseggiana*, *P. micrantha* and *P. tenuifolia* were pin-monomorphic. *P. brachybotrya* was assumed to be pin-monomorphic following Orians (1997), although we could not observe five individuals on BCI. According to Hamilton (1990), all of the pin-monomorphic species except *P. hoffmannseggiana* have both morphs elsewhere in Panama. The molecular phylogeny suggested that the three completely monomorphic species (i.e. *P. brachiata*, *P. racemosa* and *P. hoffmannseggiana*) have lost heterostyly independently (Fig. 1).

FRUIT SET OF OPEN AND BAGGED FLOWERS

Fruit set of heterostylous species varied from 5 to 43% (Fig. 2). Differences in fruit set between morphs of heterostylous species were not significant ($P > 0.05$, two-tailed *t*-test with Dunn–Sidak adjustment). Fruit set of monomorphic species ranged from 50 to 76%, and averaged 2.9 times higher than that of heterostylous species. The difference was significant (two-tailed Wilcoxon two-sample test, $P = 0.0034$).

All of the heterostylous species set few fruits when flowers were bagged. The fruit set was 2% or lower, and was 2–10% of that of open flowers (Fig. 3). In contrast, fruit set of bagged flowers of monomorphic species was 24–65%, which was 32–78% of open flowers (Fig. 3). Relative fruit set of bagged flowers was significantly higher in monomorphic species than in heterostylous species (two-tailed Wilcoxon two-sample test, $P = 0.0119$).

POLLINATORS

Five or more visitors were recorded for 19 *Psychotria* species, and a total of 559 insects with pollen loads

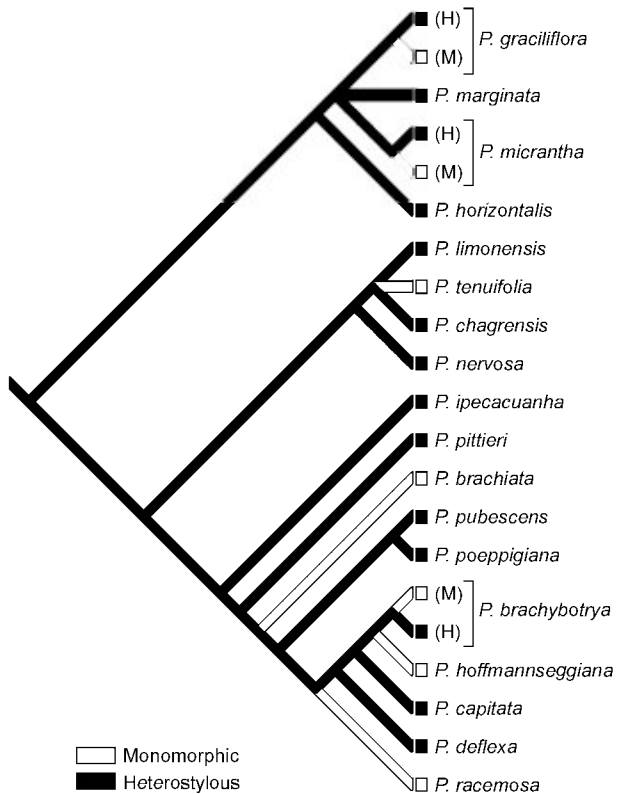


Figure 1. Estimated evolutionary changes of sexual systems using strict consensus tree based on the internal transcribed spacer region (ITS) of nuclear ribosomal DNA (Nepokroeff *et al.*, 1999). In the case of species that are monomorphic on BCI but heterostylous in other populations, the two types of populations are assumed to be monophyletic. The monomorphic and heterostylous populations are indicated by ‘M’ and ‘H’, respectively.

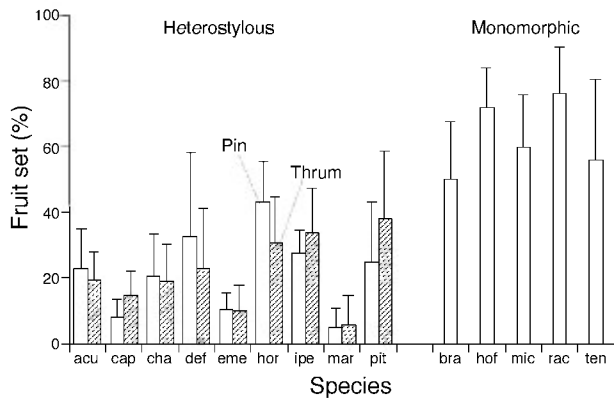


Figure 2. Fruit set 40 days after peak flowering in *Psychotria* species on BCI. Fruit set of monomorphic species is significantly greater than that of heterostylous species ($P = 0.0034$). Species codes are listed in Table 1. Appendix S1 provides sample sizes.

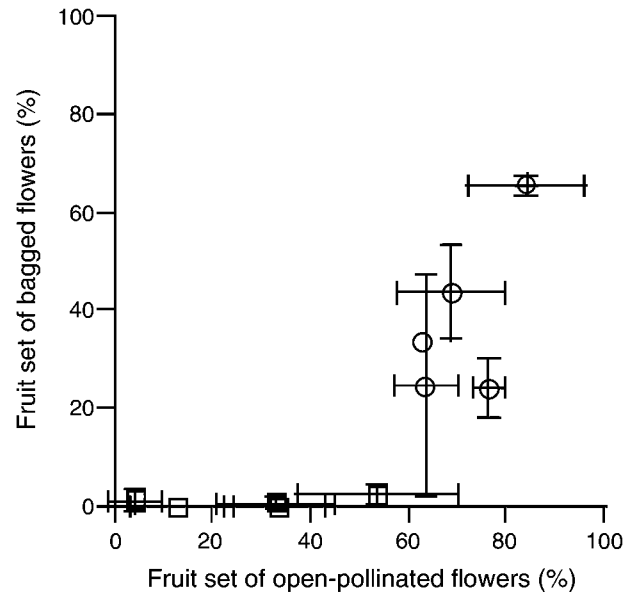


Figure 3. Plot of fruit set of bagged flowers and open flowers on the same individuals in *Psychotria* species on BCI. Circles are monomorphic species and rectangles are heterostylous species, with bars showing 1 SD. SD for *P. brachiata* was not calculated because of the small sample size. Appendix S2 provides sample sizes.

were collected (for detailed information, see Appendices S3 and S4). They mostly visited for nectar, and pollen-collecting behaviours were rarely observed. No obvious behaviours of nectar robbing, such as chewing holes at the base, were observed. There were 0.5–2 potential pollinator visits per hour in monomorphic species, with the exception of *P. micrantha* (Fig. 4A). There were 2.4–13.1 potential pollinator visits per hour in heterostylous species, with the exception of *P. emetica* (Fig. 4A). The difference between monomorphic and heterostylous species was significant (Wilcoxon two-sample test, two-tailed, $P = 0.0188$). *Psychotria poeppigiana* was the only species frequently visited by hummingbirds and euglossine bees (Fig. 5). Visitors to *P. capitata* and *P. grandis* were mostly medium sized bees, including alien African honey bees (*Apis mellifera*) and native *Megachile* and *Melipona* bees (Fig. 5). In the other 16 species, small bees accounted for 72–100% of all visits (Fig. 5). Among 424 small bees, 39% were *Paratetrapedia* spp., 21% *Osiris* spp., and 24% *Trigona* spp. The Kendall coefficient of concordance demonstrated that the species composition of small bee visitors was significantly similar among plant species ($P < 0.0001$).

PSYCHOTRIA ABUNDANCE

The 297 plots included 4243 *Psychotria* stems. Abundance varied greatly among species (for exact

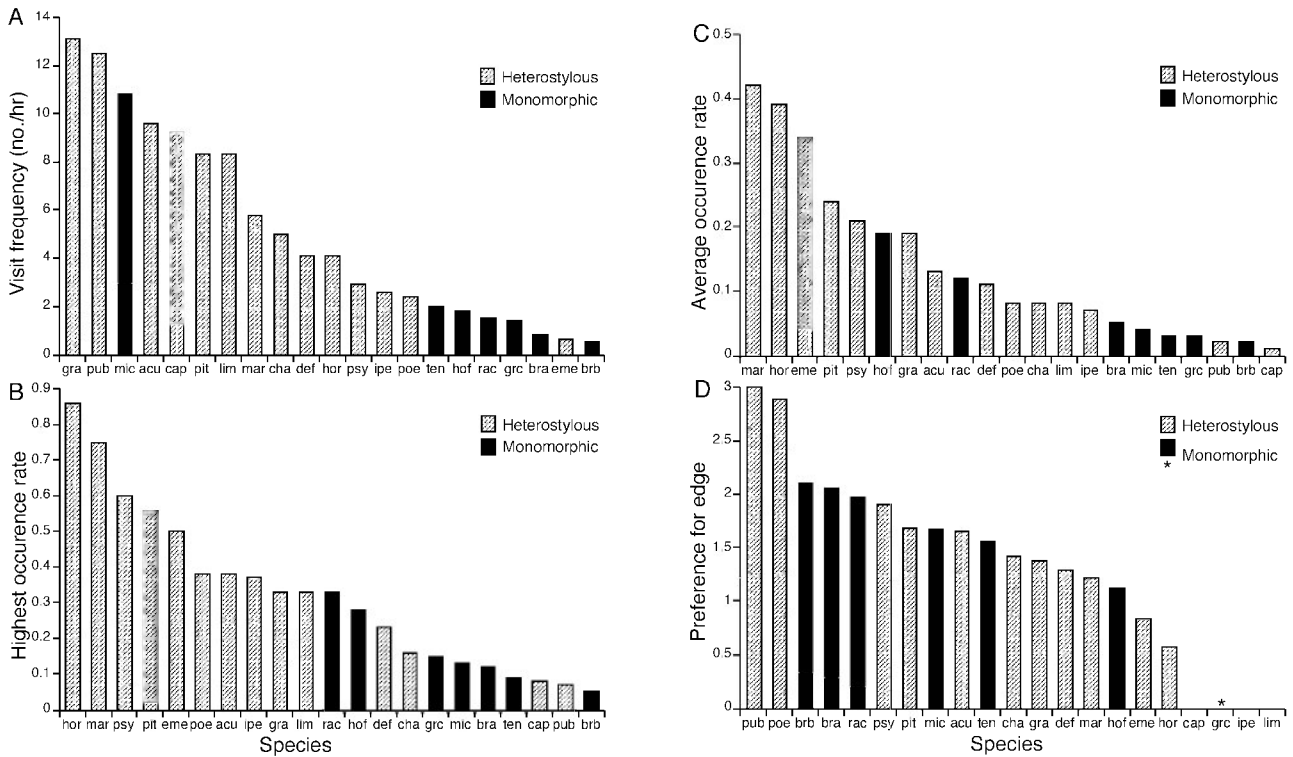


Figure 4. A, visit frequency of pollinators to *Psychotria* species at BCI and PNS. Species within the graph are ordered by frequency. Monomorphic species are less frequently visited than heterostylous species ($P = 0.0094$). B–D, the highest (B) and average (C) occurrence rate and preference for edge (D) of 21 *Psychotria* species at BCI and PNS. The former two indices are significantly lower in monomorphic species than in heterostylous species [$P = 0.008$ (B) and 0.047 (C)], while the difference in the latter is not significant (D). Species codes are listed in Table 1. Species within the graphs are ordered by each variable. Appendix S3 provides sample size for (A), and Appendix S4 provides raw data for (B–D).

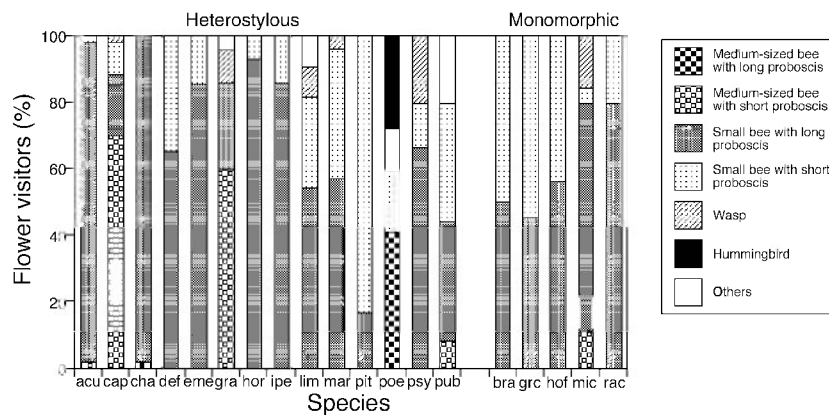


Figure 5. Proportion of different categories of pollinators of *Psychotria* species at BCI and PNS. Species codes are listed in Table 1, and pollinator species are listed in Appendix S4.

occurrence rates in each habitat see Appendix S5). *Psychotria horizontalis* occurred in 86% of the plots in moist habitat on BCI, while *P. capitata* was found in only five plots among 297. The two indices of abundance, the maximum and average of the occurrence probabilities for the six habitats, were lower in mono-

morphic species; the differences were significant and marginally significant, respectively (Fig. 4B, C; two-tailed Wilcoxon two-sample test, $P = 0.016$ and 0.094 , respectively). Preference for edge did not differ significantly between monomorphic and heterostylous species (Fig. 4D; $P > 0.05$).

DISCUSSION

Seven of the 21 study species of *Psychotria* in central Panama were monomorphic. Heterostyly has been reported outside central Panama for four of these species (*P. brachybotrya*, *P. graciliflora*, *P. micrantha*, *P. tenuifolia*; Hamilton, 1990; Orians, 1997), indicating that monomorphy evolved independently in all four species. A molecular phylogeny (Nepokroeff *et al.*, 1999; Fig. 1) confirmed that the three species that are probably monomorphic throughout their ranges also lost heterostyly independently. The frequent breakdown of heterostyly into monomorphy has been reported in other genera (Ganders, 1979; Barrett, 1988, 1992; Schoen *et al.*, 1997). However, we did not find any evidence of transition from heterostyly to dioecy, although it is considered to be a possible pathway of evolution (Bawa, 1980); fruit set was not different between the morphs in any heterostylous species.

Heterostylous and monomorphic species of *Psychotria* showed different levels of selfing. Heterostylous species set few fruits when their flowers were bagged, while monomorphic species maintained high levels of fruit set in bagged flowers (Fig. 3). Although we cannot exclude the possibility of apomixis, pollen grains were observed on the stigma of bagged flowers, indicating that pollen is transferred to the stigma without pollinators through some unknown mechanism (S. Sakai, pers. observ.); they may have a mechanism of autonomous autogamy. Open-pollinated control flowers also had much greater fruit set in monomorphic species than in heterostylous species (Fig. 2), despite lower visit frequencies by potential pollinators (Fig. 4A). Both observations suggest that monomorphic species depend more on selfing than do heterostylous species.

The results indicate that changes from heterostyly to monomorphy observed among *Psychotria* species are not simple quantitative changes in morph frequencies but are qualitative changes from outcrossing to selfing associated with the evolution of autonomous autogamy and/or the relaxation of self-incompatibility. The selective advantage associated with outcrossing can be lost when outcrossed pollen is insufficient to ensure seed production (Jain, 1976; Schoen *et al.*, 1996). Outcrossed pollen may be insufficient during population bottlenecks associated with long-distance dispersal and colonization events. Island populations illustrate the possible breakdown of heterostyly as a result of long-distance dispersal and colonization. Brazilian populations of *Eichhornia paniculata* are tristylous, while conspecific Jamaican populations have lost one or two flower types and have much lower genetic diversity (Glover & Barrett, 1987; Husband & Barrett, 1991). However, colonization effects are unlikely to

explain the breakdown of heterostyly in the *Psychotria* because four of the species that are monomorphic at the study site (*P. brachybotrya*, *P. graciliflora*, *P. micrantha* and *P. tenuifolia*) have heterostylous populations nearby and more or less continuous distributions. Parapatric distributions of monomorphic and heterostylous populations are also known from other Rubiaceae shrubs (Naiki & Nagamasu, 2004).

Outcrossed pollen may also be insufficient because of a paucity or instability of pollinator services. For example, the intensity of pollinator activity contributes to the spread and maintenance of predominantly autogamous variants of *Primula* (Primulaceae) within mostly heterostylous and outcrossing populations (Piper, Charlesworth & Charlesworth, 1984; Curtis & Curtis, 1985; Piper, Charlesworth & Charlesworth, 1986; Boyd, Silvertown & Tucker, 1990; Washitani *et al.*, 1994; Washitani, 1996). Likewise, fruit set was significantly higher for autogamous plants than for self-incompatible pin or thrum plants when unfavourable weather limited pollinator activity in *P. vulgaris* (Piper *et al.*, 1984). The repeated evolution of self-pollination may also have been associated with unreliable pollinators in *Linanthus* (Polemoniaceae), a monomorphic group (Goodwillie, 1999). Unreliable pollinators may not explain the loss of heterostyly for the *Psychotria*, because heterostylous and monomorphic species are sympatric and are visited by similar species of potential pollinators. Specifically, small bees were the predominant flower visitors for 16 of the 19 species examined, and the species composition of the small bee visitors was significantly similar among heterostylous and monomorphic species. Monomorphic species tended to receive fewer visits per hour than did heterostylous species (Fig. 4A). This might be because monomorphic species were less attractive for visitors as a result of smaller floral displays and/or rewards rather than lower activities or population sizes of their pollinators.

The density of reproductive individuals may also limit the availability of outcrossed pollen (Murawski & Hamrick, 1991, 1992; Bosch & Waser, 1999; Knapp, Goedde & Rice, 2001). Competition for pollinators with other plant species may reduce effective pollen transfer, especially in low-density plant populations (Grant, 1971). The effects of low density may be particularly pronounced for heterostylous plants because just half of the population (individuals of the other morph) can contribute pollen. In contrast, most conspecific plants are potential pollen donors in species with multi-allelic incompatibility. The significant correlation between abundance and mating systems observed for the *Psychotria* supports the hypothesis that low population density and limited pollen availability led to the loss of heterostyly. There is some indication that low abundance may be offset

by attractive flowers. The two heterostylous species with the lowest abundance, *P. capitata* and *P. pubescence*, had the second- and fifth-highest rate of flower visits by potential pollinators (Fig. 4A, C). The strong correlation between mating system and maximum abundance (Fig. 4B) also suggests that local high-density patches may be important for the maintenance of heterostyly, even when abundance over the landscape is low. A correlation between population density and self-incompatibility has also been observed in other plant species (e.g. Kunin & Shimada, 1997; Fausto, Eckert, & Geber, 2001).

An association between population density and mating system might overturn the conclusion that outcrossing predominates among tropical forest plants (Bawa, Perry & Beach, 1985; Murawski, 1995). Federov (1966) speculated that low population density should lead to high levels of self-fertilization, small population sizes, and a potential for speciation through genetic drift among tropical forest trees. Since then, rather high outcrossing rates and mechanisms ensuring outcrossing have been documented for many tropical forest plants (e.g. Hamrick & Murawski, 1990; Murawski & Hamrick, 1991; Bawa, 1992; Murawski & Hamrick, 1992; Kress & Beach, 1994; Murawski, Dayanandan & Bawa, 1994; Stacy *et al.*, 1996; Sakai *et al.*, 1999; Kenta *et al.*, 2002). The plant species in these studies are likely to be biased toward relatively abundant species because it is difficult to secure sufficient sample sizes for rare species. We might be misled if we study only common species to understand diverse tropical forests. For example, one of the interesting questions in the *Psychotria* spp. which remains to be answered is why monomorphic species are rare despite a much higher fruit set. Many ecological traits differ among common and rare plants (Gaston & Kunin, 1997). Selfing and asexual reproduction (Thomas, 1997) may turn out to be more important in tropical forests when more rare plant species are studied.

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SUPPLEMENTARY MATERIAL

The following material is available for this article online:

Appendix S1. Sample sizes for fruit set in Fig. 2.

Appendix S2. Sample sizes for fruit set of bagged and open flowers in Fig. 3.

Appendix S3. Observation (of birds) and collection (insects) time (h) and numbers of collected insects for pollinator identification in Figs 4A and 5.

Appendix S4. Numbers of flower visitors collected (in the cases of insects) or observed (in the cases of birds) on *Psychotria* species at Barro Colorado Island (BCI) and the nearby Parque Nacional Soberania (PNS). Only visitors with pollen load and visits effective for pollination are included.

Appendix S5. Occurrence rates (proportion of plots present) of 21 *Psychotria* species in six habitats on Barro Colorado Island (BCI) and the nearby Parque Nacional Soberania (PNS).

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