Population-level variation in the expression of heterostyly in three species of Rubiaceae: does reciprocal placement of anthers and stigmas characterize heterostyly?[1]

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Heterostyly (i.e., reciprocal placement of anthers and stigmas between two or three floral morphs) is hypothesized to enhance outcrossing and reduce selfing. However, few studies have documented reciprocity among individual plants; instead, mean anther and stigma heights for floral morphs are usually reported, masking interindividual variation. We measured eight floral dimensions for individuals in five populations of three heterostylous Rubiaceae. The three methods used to quantify reciprocity yielded different conclusions regarding the degree to which populations conformed to expectations for heterostylous plants. Only *Psychotria poeppigiana* had stigma and, to a lesser degree, anther heights in discrete classes. Variation among plants of *Bouvardia ternifolia* and *Psychotria chiapensis* yielded a continuum of anther and stigma heights across populations. Comparison of distances between stigma and anthers indicated that only flowers of *B. ternifolia* had, as expected, a constant value for this distance. Finally, regression relationships between anther and stigma heights and corolla length showed that only in one population each of *B. ternifolia* and *P. poeppigiana,* and in *P. chiapensis,* was distance between anthers and stigmas the same across the range of corolla sizes for both floral morphs. Variation among these species in expression of heterostyly was not clearly linked to phylogenetic relationship or pollinator syndromes. Two approach herkogamous (AH) species were studied for comparison. Flowers of *Psychotria brachiata* were consistently AH, but flowers of *P. pittieri* were highly variable. Determining fitness consequences of population-level variation in sexual systems requires studies linking floral morphology to pollinator behavior and pollen transfer.

Key words: approach herkogamy; *Bouvardia*; distyly; floral morphology; heterostyly; plant sexual systems; *Psychotria*; Rubiaceae.

Heterostyly is in part defined as the reciprocal placement of stigma and anthers in two (distyly) or three (tristyly) floral morphs of one species (Darwin, 1877; Ganders, 1979; Lloyd and Webb, 1992a). Individual plants of distylous species produce either all long-styled (LS) or all short-styled (SS) flowers. Flowers with the LS morphology have stigma(s) positioned above anthers, whereas flowers with the SS morphology have anthers above stigma(s). In addition to differences in floral morphology, heterostylous species typically have a genetically based incompatibility system that prevents fertilization following self and intramorph pollination (i.e., between different individuals with flowers of the same morphology) (Barrett, 1990, 1992). Heterostyly has been documented in 28 angiosperm families (Barrett, Jesson, and Baker, 2000), and phylogenetic information indicates that it has evolved independently in at least 23 of these (Lloyd and Webb, 1992a).

The evolution and maintenance of heterostyly are thought to be driven by outcrossing advantage: selection favors reciprocal placement of stigma and anthers between morphs because it results in efficient transfer of intermorph pollen (Darwin, 1877; Lloyd and Webb, 1992b). Charlesworth and Charlesworth (1979b) hypothesized more specifically that heterostyly would evolve in populations with high levels of inbreeding depression and that evolution of the heterostylous incompatibility system would precede establishment of reciprocal positioning of anthers and stigmas between morphs. To evaluate these evolutionary models, it is essential to determine the degree to which heterostylous plants actually present the "typical" heterostylous syndrome. In terms of morphology, flowers on heterostylous plants should be of two classes corresponding to LS and SS flowers as described above, with little variation among flowers of the same morph. Further, the absolute value of the difference between anther and stigma heights should be identical between floral morphs. That is, there should be strict reciprocity between heights of these organs in LS and SS flowers.

These aspects of heterostyly have rarely been addressed explicitly, in part because data from different individuals are usually pooled and analyzed as a comparison between morphs. When pooled, mean stigma and anther heights are usually significantly different between morphs (Sobrevila, Ramírez, and de Enrech, 1983; Passos and Sazima, 1995; Riveros, Barría, and Humaña, 1995; Nishihiro and Washitani, 1998), with some exceptions (Richards and Koptur, 1993; Pailler and Thompson, 1997). However, these studies do not address degree of variation among flowers or individuals of the same morph (but see Richards and Koptur, 1993; Eckert and Barrett, 1994; Pailler and Thompson, 1997).

In this paper, we document patterns of expression of het-

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erostyly in five populations of three species of Rubiaceae. Rubiaceae have more heterostylous species than any other plant family, reflecting several origins of the syndrome as noted by Darwin (1877) and Bahadur (1968). Our goals were to examine variation among individuals with regard to key floral dimensions, as well as to document degree of reciprocity among individuals within populations. In addition, the species studied vary in degree of relatedness (two *Psychotria* species, one *Bouvardia*) and in pollination syndrome (hummingbird vs. hawk moth), permitting an initial exploration of the source(s) of variation in the expression of heterostyly. If floral morphological variation has a strong phylogenetic component, we expect the two species of *Psychotria* to be most similar; alternatively, flowers of the two hummingbird-pollinated species should be most similar if pollinators have been important agents of selection. Finally, we compare the heterostylous species to two psychotrias with approach herkogamous (AH) flowers (i.e., stigmas positioned above anthers; Webb and Lloyd, 1986). Flowers of AH species should be under selection to maintain stigma height above anthers but not for precise positioning of these organs compared to conspecifics. Variation within and among individuals of species with AH flowers should thus provide a basis for evaluating variation found among the heterostylous taxa in floral morphology. In addition, approach herkogamy has been proposed as the evolutionary precursor to heterostyly (Lloyd and Webb, 1992b); if this is the case, then patterns of morphological variation in these flowers may offer insight into the evolutionary path(s) to heterostyly.

MATERIALS AND METHODS

Study taxa and sites—The heterostylous species studied were subtropical *Bouvardia ternifolia* and two tropical species of *Psychotria* (*P. poeppigiana* and *P. chiapensis*); the two approach herkogamous (AH) species were also tropical psychotrias (*P. brachiata* and *P. pittieri*). Ideally, the AH species would be closely related to the heterostylous species. A species-level phylogeny of *Psychotria* is not available, but the results of Nepokroeff, Bremer, and Sytsma (1999) indicate that three of the four psychotrias are members of the neotropical "subgenus *Heteropsychotria* plus *Palicourea*" clade. (*P. chiapensis* was not included in this analysis but is thought to be a member of this clade as well [C. Taylor, Missouri Botanical Garden, personal communication].) However, *P. poeppigiana* and *P. chiapensis* are probably not closely related within that clade as the former was placed in *Cephaelis,* a genus of some 100 species now synonymized with *Psychotria* (Steyermark, 1972). *Bouvardia ternifolia* is more distantly related, although it is in the same subfamily (Rubioideae) as *Psychotria*.

Flowers of *P. poeppigiana* and *B. ternifolia* were predominantly visited by hummingbirds, butterflies, and small bees (A. Faivre, unpublished data). The bright red flowers of *B. ternifolia* presumably attracted pollinators, whereas *P. poeppigiana* has large, red bracts surrounding inflorescences of small (~15 mm in length), yellow flowers. *Psychotria chiapensis* bloomed at night, and flowers were visited predominantly by hawk moths and perching moths (A. Faivre, unpubl. data). The small, yellow diurnal flowers of the two approach herkogamous species, *P. brachiata* and *P. pittieri,* were visited by small bees and wasps (A. Faivre, unpublished data).

Plants of *Bouvardia ternifolia* form a contiguous population extending for >1 km along Molino Basin, a drainage in the Santa Catalina mountains (SCM) northeast of Tucson, Arizona (1310–1470 m elevation; 32°17′ N, 110°40′ W). In 1994 and 1995, flowers were collected from 25 plants that produce long-styled (LS) flowers and 25 that produce short-styled (SS) flowers. These plants flowered from late April through late September, with flowering peaks in late May–early June and August. The second study population of *B. ternifolia* was located in Florida Canyon, Santa Rita Mountains (SRM), ~60 km south of Tucson (1305–1340 m elevation; 31°45′ N, 110°50′ W).
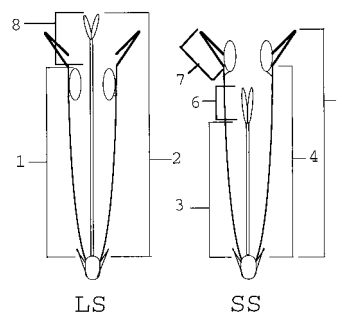

Fig. 1. Long-styled (LS) and short-styled (SS) flowers of *Bouvardia ternifolia*. Numerals correspond to the following measurements for both floral morphs: (1) anther height, (2) stigma height, (3) style height, (4) corolla tube length, (5) corolla length, (6) stigma length, (7) petal length, and (8) difference between stigma and anther height.

Flowers were collected in 1997 from ten plants with LS and seven with SS flowers.

All four *Psychotria* species co-occur at La Selva Biological Station (LAS), Costa Rica (50–75 m elevation; 10°26′ N, 83°59′ W; McDade and Hartshorn, 1994). In 1995–1997 flowers of *P. poeppigiana* were collected from 11 plants with LS flowers and 24 with SS flowers from two patches ~400 m apart on the Atajo and Jaguar trails. Additional data were gathered in July 1997 from a population of *P. poeppigiana* along "Pipeline Road" in Soberanía National Park (SNP), Panama (25–35 m elevation, 9°9′ N, 79°51′ W; Croat, 1978). Data were collected from eight plants that produce LS flowers and nine with SS flowers. Both populations of *P. poeppigiana* appeared to flower throughout the year as the result of asynchronous, but overlapping, flowering episodes of individual plants (Burger and Taylor, 1993).

Flowers of *P. chiapensis* were collected from a total of 11 plants with LS flowers and 10 with SS flowers on the Tres Ríos trail at La Selva. At LAS, plants of *P. chiapensis* flower from May to September and in January (Burger and Taylor, 1993).

Flowers were gathered from six plants each of *P. brachiata* and *P. pittieri* along the Atajo trail in July of 1996 and from seven plants of *P. brachiata* and six of *P. pittieri* along the Holdridge trail in July and August of 1997. Plants of *P. brachiata* flower throughout the year, with peak flowering at LAS from May to July (Burger and Taylor, 1993); peak flowering of *P. pittieri* also occurs from May to July (Burger and Taylor, 1993).

Floral phenology and morphology—Whereas the four *Psychotria* species produce flowers that are open for 1 d, flowers of *B. ternifolia* remain open for 5 d on average (range = 3–8 d; A. Faivre, unpublished data). After the first day of anthesis, the corolla lobes change from bright red to a duller red or maroon (A. Faivre, unpublished data). The color change facilitated distinguishing newly opened flowers from those that had been open >1 d. Only first-day flowers of all species were measured, with the exception of the SRM population of *B. ternifolia*. Here, flowers were gathered regardless of age, but were scored as first day or older. Flowers from all populations were measured immediately after collection or were refrigerated in glass vials and measured within 72 h of collection. No changes were observed in refrigerated flowers, except that some *Bouvardia* flowers from the SRM population had slightly wilted. This was noted, but analyses indicated that post-collection changes did not affect results.

For measurement, corollas were slit longitudinally from just above the inferior ovary to the mouth. The apex of the inferior ovary was the baseline for all measurements (Fig. 1). Calipers were used to measure: (1) anther height (stamens are epipetalous with minimal filaments), (2) stigma height (with stigma lobes closed and held vertically), (3) style height (not including the stigma), (4) corolla tube length, and (5) corolla length (numerals in Fig. 1 correspond to dimensions measured). (6) Stigma length was calculated by subtracting style height (3) from stigma height (2). (Style height was used only to calculate stigma length and will not be discussed further.) (7) Petal length, likewise, was calculated by subtracting corolla tube length from the

length of the entire corolla. The difference between stigma and anther heights (8) was calculated for each flower as the absolute value of anther height less stigma height.

Analyses—All analyses were conducted in SAS (SAS, 1990). For each population, variation among individuals of the same floral morph was assessed for the eight characters described above and in Fig. 1. Means for individual LS and SS plants were compared using one-way ANOVA. Pairwise *t* tests were used to conduct an a posteriori multiple comparison of means for individual plants. To assess variation among individuals regardless of floral morph, data for all plants (i.e., both LS and SS morphs) from each population were analyzed using the same tests. The two floral morphs in each population were compared using means of the mean values for each individual for each floral dimension. Means were used because sample sizes (i.e., number of flowers per individual) varied considerably for *B. ternifolia* from SCM such that data from plants from which many flowers were measured would have unduly influenced the results if means for the two floral morphs had been calculated from data for all flowers. Wilcox two-sample tests (χ^2 approximation) were used to test for differences between floral morphs for each variable measured.

Comparison of the absolute value of anther height less stigma height permits an assessment of reciprocity: if floral organs are positioned reciprocally between floral morphs, then these values should be constant among individuals and between morphs. However, as for essentially all Asteridae, flowers of these plants have epipetalous stamens such that anther height may track variation in corolla length among flowers. We therefore used an additional approach to incorporate any relationship between corolla length and anther (or stigma) height into our assessment of reciprocity between anther and stigma height. The relationships between mean corolla length and mean anther and stigma heights per plant were described for each floral morph using linear regression. Slopes that differed significantly from zero indicated a relationship between corolla length and the corresponding floral organ. For the difference between stigma and anther heights to remain constant among flowers of the same floral morph that vary in corolla length, relative change in anther and stigma heights with changing corolla length (i.e., slopes of the regression relationships) must be equivalent (Fig. 2a). When the slopes are significant but unequal, height of one floral organ increases more than the other (Fig. 2b). Slopes of the regression lines for mean anther and stigma heights for plants of the same floral morph were thus compared within populations using the ANCOVA homogeneity of slopes model.

RESULTS

Intramorph variation—In all heterostylous populations, plants of the same floral morph showed a range of anther and stigma heights (Figs. 3–5), with significant differences among some plants (for details see appendices in Faivre, 1998). There were significant differences among plants of the same floral morph for essentially all other floral dimensions measured (exceptions are petal length [both populations] and stigma lobe length [SNP] of *P. poeppigiana* which did not vary among individuals of the same morph) (results not shown). However, results of the a posteriori *t* tests indicated that no plant from any population had flowers that differed significantly in all dimensions from those of other plants of the same floral morph.

Variation between long-styled and short-styled flowers—For all three distylous species in all populations studied, anther heights of SS flowers significantly exceeded those of LS flowers, and stigma heights in LS flowers significantly exceeded those of SS flowers. For all other floral dimensions that were compared between LS and SS flowers, however, there were differences among species and in some cases between populations, as described below.
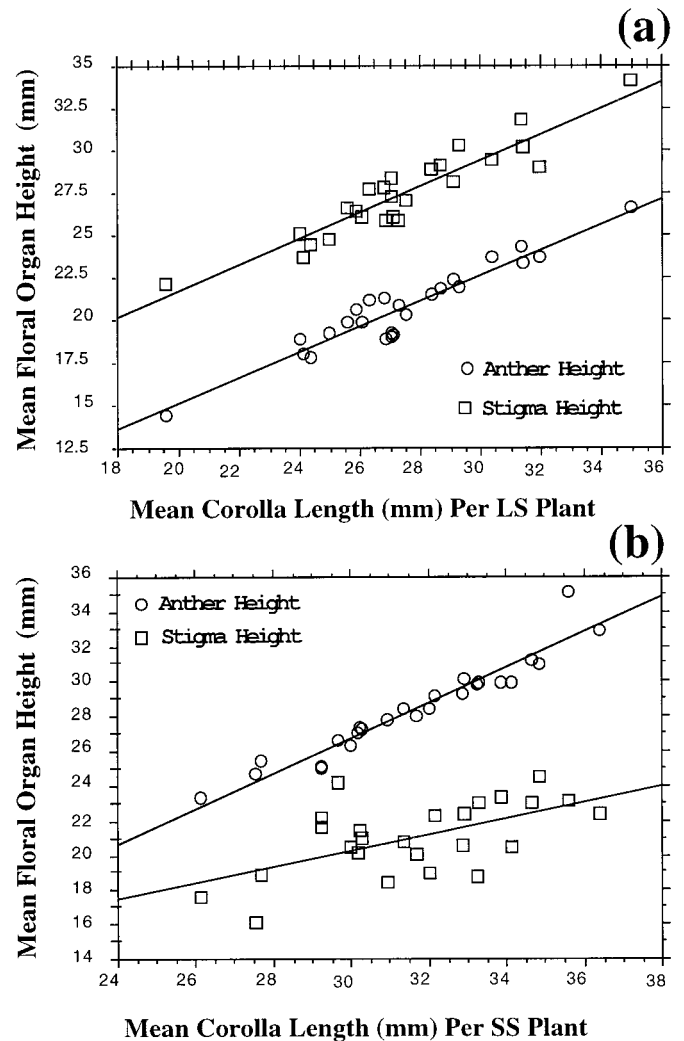

Fig. 2. Relationship between floral organ height and corolla length in flowers of *Bouvardia ternifolia* from the Santa Catalina Mountains. (a) Plants of the LS morphology. Mean anther heights (circles) and mean stigma heights (squares) per plant are plotted against mean corolla length per plant. (b) Plants of the SS morphology. Mean anther heights (circles) and mean stigma heights (squares) per plant are plotted against mean corolla length per plant.

Bouvardia ternifolia—Short-styled flowers significantly exceeded LS flowers in both populations in corolla length, corolla tube length, anther height and stigma size (Table 1). There was no significant difference in petal size between morphs in either population. The ranges of mean anther and stigma heights of individuals with LS flowers overlapped those of individuals with SS flowers in SCM (Fig. 3a, b) and in SRM (Fig. 3d, e).

In both populations, the difference between stigma and anther heights within flowers did not differ between floral morphs (Table 1). However, this distance was highly variable among individuals (Fig. 3c, f; see also CVs, Table 1). This distance was determined by both anther and stigma height and yet was not clearly associated with either of these values. That is, plants are ordered by increasing anther height in Fig. 3a–f (note that Fig. 3b and e indicate that this order was similar but not identical for stigma heights). In contrast, the magnitude of the difference between anther and stigma heights (Fig. 3c,
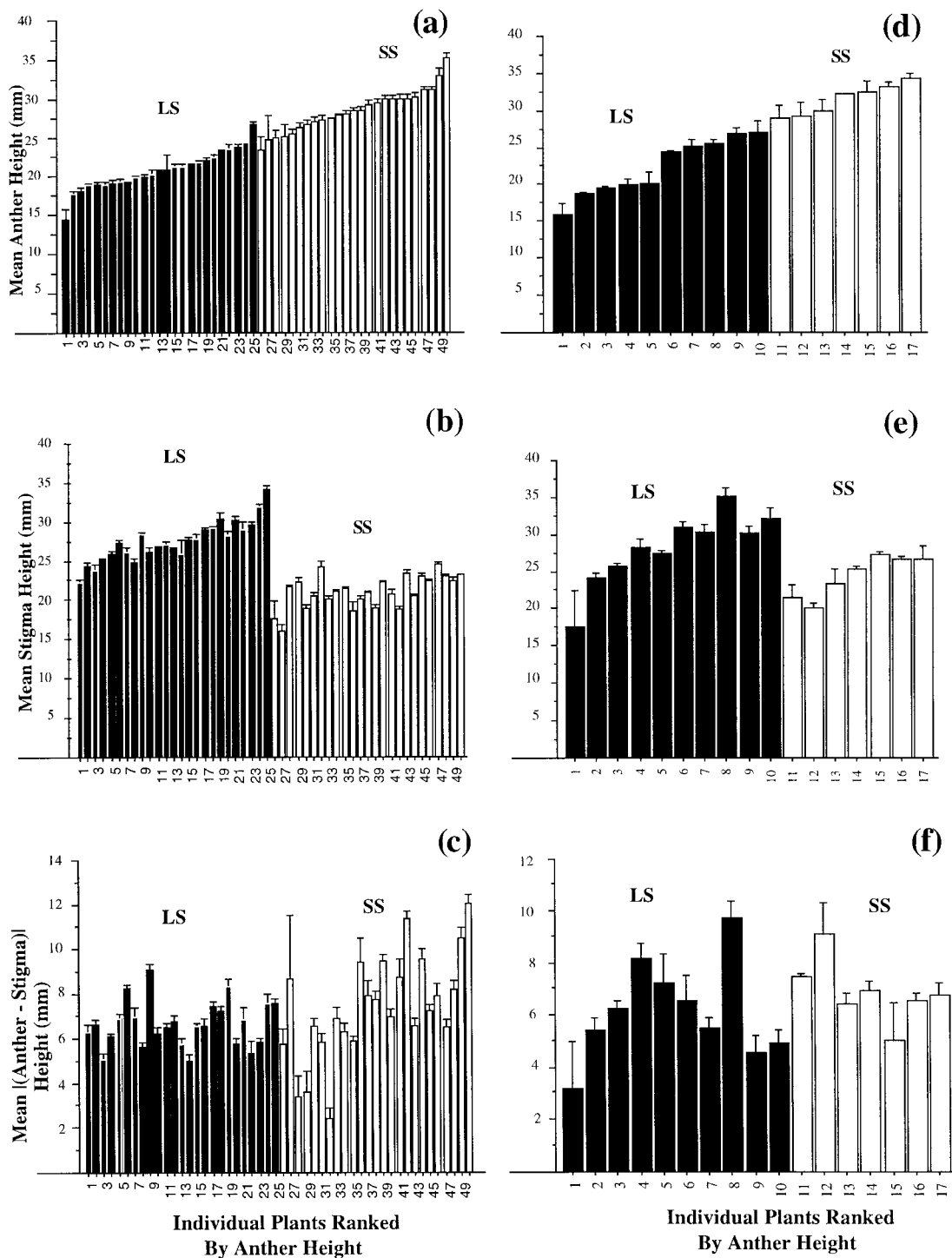
Fig. 3. Floral morphology of plants of *B. ternifolia,* Santa Catalina Mountains (a–c) and Santa Rita Mountains (d–f). Bars represent mean height per plant + 1 SE. Shaded bars represent plants with long-styled flowers (LS), unshaded bars represent plants with short-styled flowers (SS). (a, d) Anther heights of LS and SS plants. (b, e) Stigma heights of LS and SS plants. (c, f) Absolute value of difference between anther and stigma heights within individual flowers for LS and SS plants.

f) varied across individuals with no correspondence to either anther or stigma heights.

Psychotria poeppigiana—In both populations, stigma lengths of SS flowers exceeded those of LS flowers. Corolla length did not vary between floral morphs of *P. poeppigiana*

at LAS, but LS flowers had longer petals than SS flowers (Table 2). In contrast, at SNP, SS flowers had significantly longer corollas and corolla tubes than LS flowers (Table 2). (Note that there was no difference between floral morphs in petal length in this population, indicating that differences in corolla length were due to corolla tube length.) Mean anther
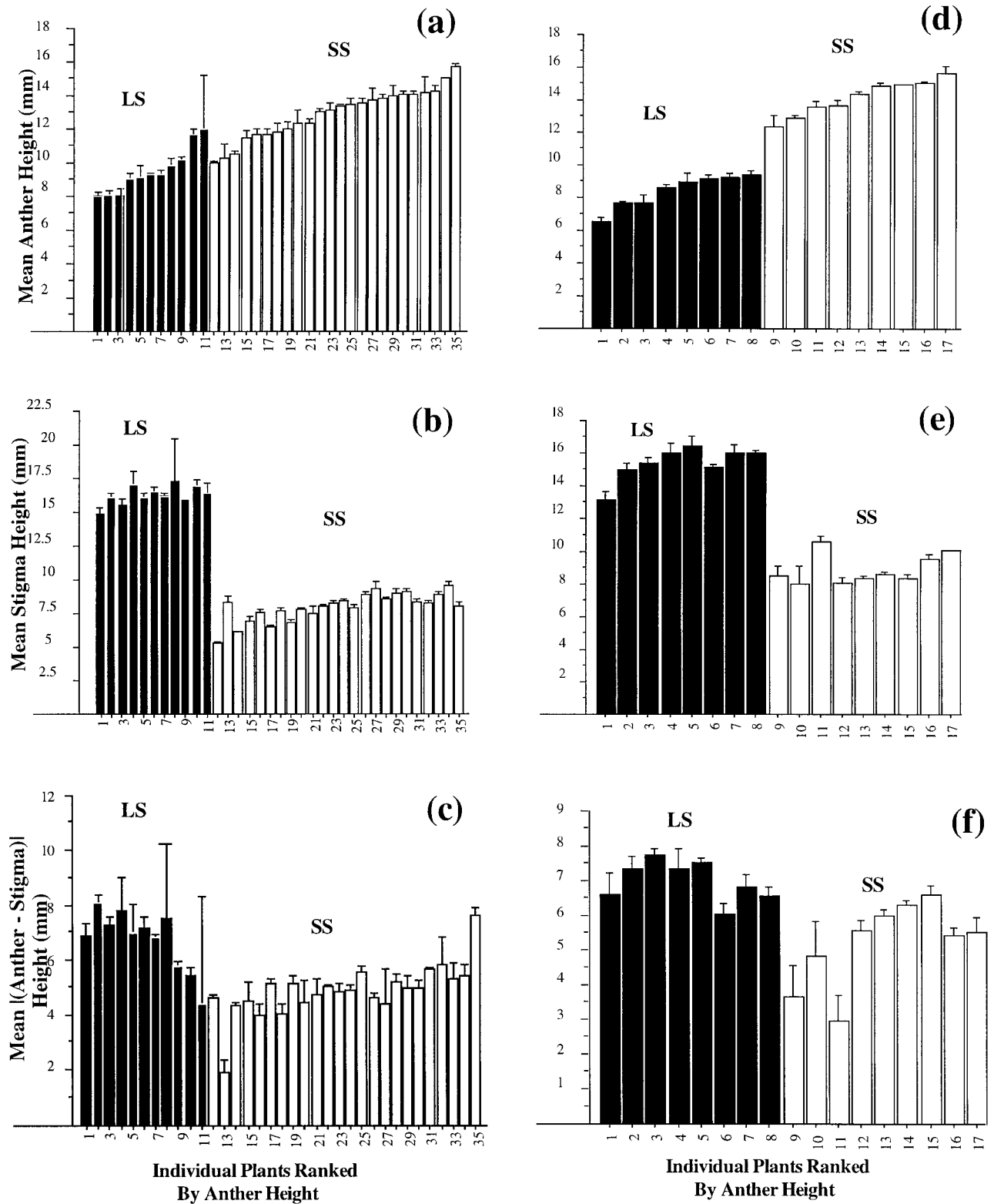
Fig. 4. Floral morphology of plants of *P. poeppigiana,* La Selva (a–c) and Soberanía National Park (d–f). Bars represent mean height per plant + 1 SE. Shaded bars represent plants with long-styled flowers (LS), unshaded bars represent plants with short-styled flowers (SS). (a, d) Anther heights of LS and SS plants. (b, e) Stigma heights of LS and SS plants. (c, f) Absolute value of difference between anther and stigma heights within individual flowers for LS and SS plants.
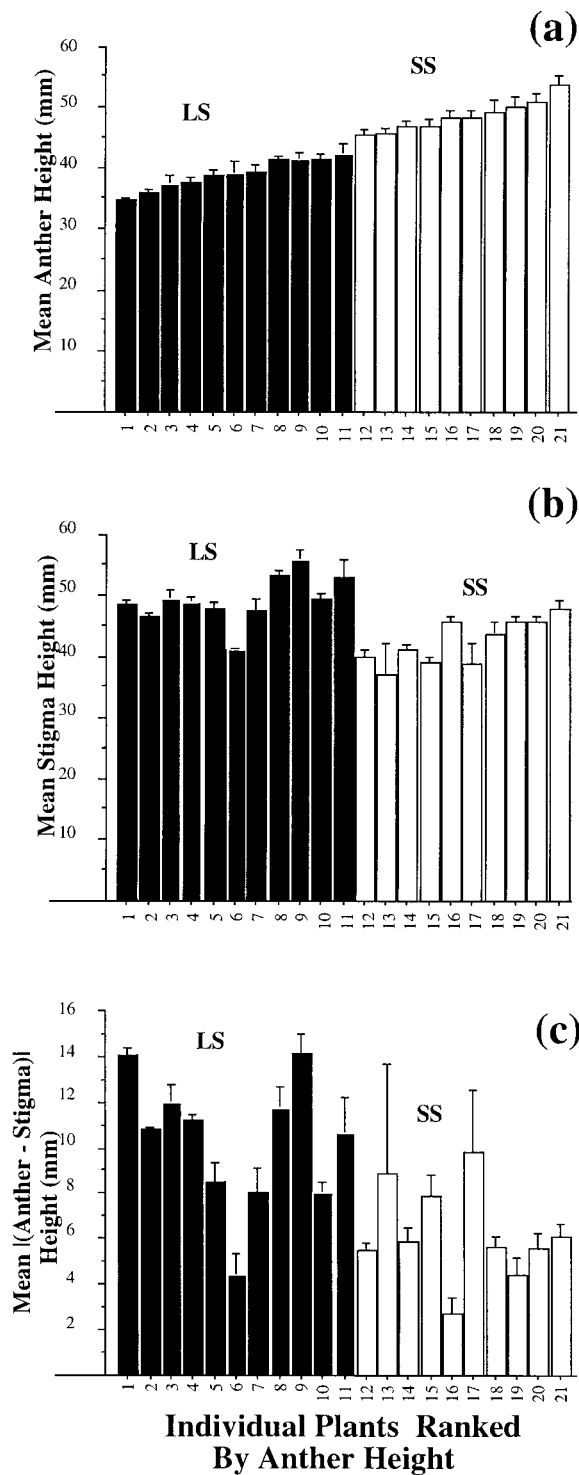
Fig. 5. Floral morphology of plants of *P. chiapensis,* La Selva. Bars represent mean height per plant + 1 SE. Shaded bars represent plants with long-styled flowers (LS), unshaded bars represent plants with short-styled flowers (SS). (a) Anther heights of LS and SS plants. (b) Stigma heights of LS and SS plants. (c) Absolute value of difference between anther and stigma heights within individual flowers for LS and SS plants.

TABLE 1. Floral dimensions (in millimeters) and coefficients of variation (CV) for plants of long-styled (LS) and short-styled (SS) morphs of *Bouvardia ternifolia* from the Santa Catalina (SCM) and Santa Rita (SRM) Mountains. Note that means represent mean values calculated for each individual. The range of flowers measured per individual was 2–48 for LS and 2–34 for SS plants from SCM and 2–8 (LS) and 2–6 (SS) plants from SRM. $*P < 0.05$, $**P < 0.01$, $***P < 0.0001$, ns = not significant.

| | SCM | | | | | | SRM | | | | | |
| | LS (25)[b] | | | SS (25)[b] | | | LS (10)[b] | | | SS (7)[b] | | |
Floral dimension[a]	Mean ± SD	CV (%)	No. flowers measured	Mean ± SD	CV (%)	No. flowers measured	Mean ± SD	CV (%)	No. flowers measured	Mean ± SD	CV (%)	No. flowers measured
1 Anther height	20.7 ± 2.5	12.1	309	28.4 ± 2.7	9.5	307***	22.3 ± 3.9	17.5	46	31.5 ± 2.1	6.7	25**
2 Stigma height	27.5 ± 2.6	9.4	301	21.0 ± 2.1	10.0	297****	28.2 ± 4.9	17.4	45	24.4 ± 2.8	11.5	21*
4 Corolla tube length	23.7 ± 2.8	11.8	310	28.3 ± 2.6	9.2	308****	24.2 ± 4.1	16.0	46	31.5 ± 2.1	6.7	25**
5 Corolla length	27.4 ± 3.1	11.3	310	31.6 ± 2.6	8.2	308****	27.5 ± 4.4	16.0	46	34.5 ± 2.2	6.4	25**
6 Stigma length	2.4 ± 0.5	20.8	300	2.7 ± 0.4	14.8	296**	2.5 ± 0.5	20.0	45	2.9 ± 0.4	13.8	21*
7 Petal length	3.8 ± 0.6	15.8	308	3.4 ± 0.5	14.7	306 ns	3.3 ± 0.5	15.1	46	3.1 ± 0.4	12.9	25 ns
8 Absolute value of anther less stigma height	6.6 ± 1.0	15.1	301	7.4 ± 2.3	31.1	296 ns	6.2 ± 1.9	30.6	45	6.8 ± 1.2	17.6	21 ns

[a] Numbers correspond to Fig. 1.
[b] Number of plants from which flowers were taken.

TABLE 2. Floral dimensions (in millimeters) and coefficients of variation (CV) for plants of the long-styled (LS) and short-styled (SS) morphs of *Psychotria poeppigiana* from La Selva Biological Station, Costa Rica (LAS), and Soberanía National Park, Panama (SNP). Note that means represent mean values calculated for each individual. The range of flowers measured per individual was 2–23 for LS and 2–13 for SS plants from LAS and 3–9 (LS) and 2–9 (SS) plants from SNP. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$, ns = not significant.

| | LAS | | | | | | SNP | | | | | |
| | LS (11)[b] | | | SS (24)[b] | | | LS (8)[b] | | | SS (9)[b] | | |
Floral dimension[a]	Mean ± SD	CV (%)	No. flowers measured	Mean ± SD	CV (%)	No. flowers measured	Mean ± SD	CV (%)	No. flowers measured	Mean ± SD	CV (%)	No. flowers measured
1 Anther height	9.4 ± 1.3	13.8	73	12.9 ± 1.5	11.6	96***	8.4 ± 1.0	11.9	45	14.1 ± 1.1	7.8	45**
2 Stigma height	16.3 ± 0.7	4.3	67	8.0 ± 1.0	12.5	96***	15.4 ± 1.0	6.5	45	8.9 ± 0.9	10.1	45**
4 Corolla tube length	12.7 ± 1.4	11.0	71	13.3 ± 1.6	12.0	95 ns	12.4 ± 1.2	9.7	45	14.5 ± 0.7	4.8	45***
5 Corolla length	15.0 ± 1.3	8.7	73	14.9 ± 1.5	10.1	92 ns	14.6 ± 1.3	8.9	45	16.5 ± 0.8	4.8	45*
6 Stigma length	1.9 ± 0.7	36.8	67	3.1 ± 0.5	16.1	96***	1.8 ± 0.7	38.9	45	3.0 ± 0.5	16.7	42**
7 Petal length	2.3 ± 0.3	13.0	71	1.7 ± 0.2	11.8	92***	2.2 ± 0.2	9.1	45	2.0 ± 0.3	15.0	45 ns
8 Absolute value of anther less stigma height	6.8 ± 1.1	16.2	67	4.9 ± 1.0	20.4	96**	7.0 ± 0.6	8.6	45	5.2 ± 1.1	21.2	42**

[a] Numbers correspond to Fig. 1.
[b] Number of plants from which flowers were taken.

heights in flowers of *P. poeppigiana* from plants at LAS formed a continuum, with overlap between LS and SS individuals (Fig. 4a). However, in the SNP population, mean anther heights were in two distinct groups corresponding to plants of the LS and SS morph (Fig. 4d). In both populations, mean stigma heights per individual were in two discrete classes corresponding to LS and SS flower morphs (Fig. 4b, e).

The difference between stigma and anther heights within individual flowers was greater for LS flowers than SS flowers. However, as for *B. ternifolia,* the distance between stigma and anther heights varied considerably among individuals (Fig. 4c, f; see CVs in Table 2) and was not clearly associated with anther or stigma heights.

Psychotria chiapensis—Short-styled flowers of *P. chiapensis* from plants at LAS exceeded LS flowers in stigma length, corolla length, and corolla tube length (Table 3). However, petal length did not differ between floral morphs. Mean anther and stigma heights in flowers of *P. chiapensis* formed a continuum with little (anther height) or considerable (stigma height) overlap between plants of the two floral morphs (Fig. 5a, b).

As with *P. poeppigiana,* the difference between stigma and anther heights within individual flowers was significantly greater in LS flowers than in SS flowers. There was considerable variation in the distance between stigma and anther heights among individuals (Fig. 5c; see CVs in Table 3) and as with the other two species this variation did not track anther or stigma heights in a straightforward way.

Psychotria brachiata and Psychotria pittieri—Mean anther and stigma heights for AH flowers of *P. brachiata* formed a continuum among plants (Fig. 6a, b), but varied little (see CVs in Table 4). Among individuals of *P. pittieri,* there was little variation in anther heights, whereas stigma heights were more variable (see CVs in Table 4). However, no plant had floral dimensions significantly different from others (Fig. 6c, d).

Corolla length, anther and stigma heights—For all heterostylous species, there was a significant relationship between mean corolla length per plant and both mean anther and mean stigma height (i.e., the slopes of the regression lines differed significantly from zero; Table 5). However, for the two AH species, only the relationship between mean corolla length and mean anther height per plant was significant (Table 5).

The slopes of the regression of mean anther and stigma heights on mean corolla length did not differ among plants of the same floral morph of one population each of *Bouvardia ternifolia* (SRM) and *Psychotria poeppigiana* (SNP) and for *P. chiapensis* (Table 5). This same pattern held for plants with LS flowers of *B. ternifolia* at SCM; however, in SS flowers, mean anther height increased more rapidly than mean stigma height with increasing corolla length (Fig. 2; Table 5). In flowers on plants of both morphs of *P. poeppigiana* at LAS, mean anther height increased more rapidly with increasing corolla length than did mean stigma height (Table 5). These results indicate that flowers of *B. ternifolia* at SCM and of *P. poeppigiana* at LAS do not have reciprocal stigmas and anthers across the range of corolla lengths found in these populations.

DISCUSSION

Intraspecific variation in floral morphology—Intraspecific variation in the traits associated with heterostyly may occur at

TABLE 3. Floral dimensions (in millimeters) and coefficients of variation (CV) for plants of the long-styled (LS) and short-styled (SS) morphs of *Psychotria chiapensis* from La Selva Biological Station, Costa Rica. Note that means represent mean values calculated for each individual. The range of flowers measured per individual was 2–18 for LS and 4–16 for SS plants. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$, ns = not significant.

Floral dimension[a]	LS (11)[b]			SS (10)[b]		
	Mean ± SD	CV (%)	No. flowers measured	Mean ± SD	CV (%)	No. flowers measured
1 Anther height	39.1 ± 2.5	6.4	81[c]	48.7 ± 2.6	5.3	85***
2 Stigma height	49.2 ± 3.9	7.9	79	42.5 ± 3.7	8.7	86**
4 Corolla tube length	41.2 ± 2.9	7.0	73	44.4 ± 2.1	4.7	76*
5 Corolla length	54.0 ± 4.2	7.8	81	58.7 ± 3.0	5.1	85*
6 Stigma length	5.1 ± 1.0	19.6	79	9.1 ± 1.0	11.0	86***
7 Petal length	12.8 ± 2.2	17.2	73	14.1 ± 1.3	9.2	76 ns
8 Absolute value of anther less stigma height	10.3 ± 2.9	28.1	79	6.2 ± 2.1	33.9	85**

[a] Number corresponds to Fig. 1.
[b] Number of plants from which flowers were taken.

several levels (e.g., among flowers on a single individual, among individuals in a population, among populations). These have different implications for both the proximate source of variation (e.g., plasticity vs. genetic control), as well as ecological and evolutionary causes. Documenting the level at which variation occurs is thus key to understanding the significance and source of this variation. Unfortunately, most studies of heterostyly group individuals by floral morph and report floral dimensions as an average for all LS and all SS flowers. Further, few have included plants from more than one population.

Data from individuals demonstrate considerable variation among plants of the same floral morph for essentially all variables measured for all three heterostylous species. The continuum of stigma and anther heights across individual plants of the same morph of *Bouvardia ternifolia* and *Psychotria chiapensis* indicates that these plants are not strictly heterostylous. That is, at the population level, heights of these floral
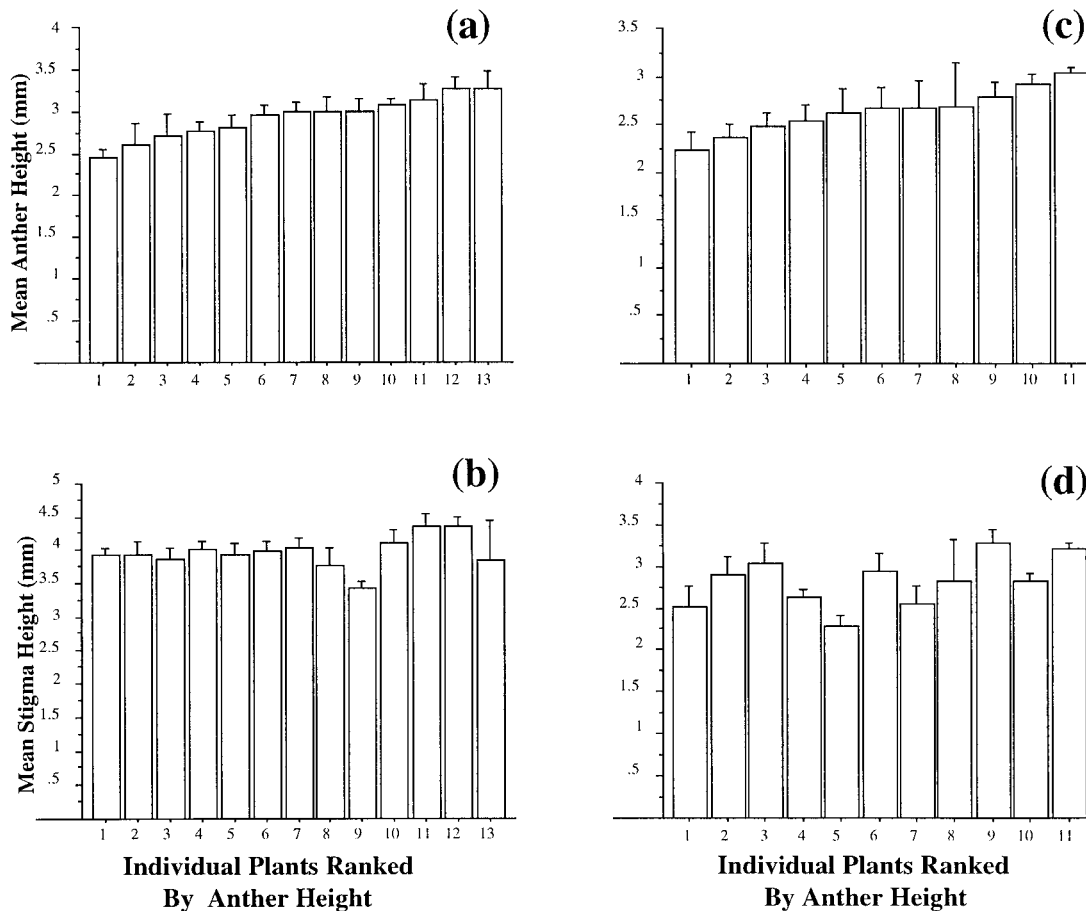

Fig. 6. Floral morphology of plants of *P. brachiata,* La Selva (a, b) and of *P. pittieri,* La Selva (c, d). Bars represent mean height per plant + 1 SE. (a, c) Anther heights of plants. (b, d) Stigma heights of plants.

TABLE 4. Floral dimensions (in millimeters) and coefficients of variation (CV) for plants of *Psychotria brachiata* and *P. pittieri* from La Selva Biological Station, Costa Rica. Note that means represent mean values calculated for each individual. The range of flowers measured per individual was 2–11 for plants of *P. brachiata* and 2–7 for plants of *P. pittieri*.

Floral dimension[a]	*P. brachiata* (13)[b]			*P. pittieri*[b]		
	Mean ± SD	CV (%)	No. flowers measured	Mean ± SD	CV (%)	No. flowers measured
1 Anther height	2.9 ± 0.2	6.9	84	2.6 ± 0.2	7.7	50
2 Stigma height	4.0 ± 0.2	5.0	82	2.8 ± 0.3	10.7	48
4 Corolla tube length	4.8 ± 0.5	10.4	86	2.8 ± 0.3	10.7	49
5 Corolla length	6.6 ± 0.5	7.6	86	3.9 ± 0.4	10.2	49
6 Stigma length	1.2 ± 0.2	16.7	81	0.8 ± 0.2	25.0	41
7 Petal length	1.8 ± 0.2	11.1	86	1.1 ± 0.2	18.7	49
8 Absolute value of anther less stigma height	1.1 ± 0.3	27.3	81	0.3 ± 0.2	66.7	48

[a] Number corresponds to Fig. 1.
[b] Number of plants from which flowers were taken.

organs were not of two distinct classes (Figs. 3, 5). Previous studies that included data for individuals have documented a similar continuum of stigma and anther heights across plants of *Guettarda scabra* (Rubiaceae) (Richards and Koptur, 1993) and *Decodon verticillatus* (Lythraceae; Eckert and Barrett, 1994). However, Richards and Koptur (1993) found distinct classes of stigma and anther heights for LS and SS flowers of *Psychotria nervosa.* More studies that track individuals will be necessary to determine the degree to which plants of heterostylous species are of two (or three) strictly distinct morphs.

With regard to between-morph variation in ancillary features of heterostyly (e.g., corolla length, stigma size), populations of both species that were represented here by two populations were fairly consistent. However, some other studies have found variation in ancillary features among populations of the same species. In two species of *Palicourea* (a genus nested within *Psychotria*; Taylor, 1996; Nepokroeff, Bremer, and Sytsma, 1999), Sobrevila, Ramírez, and de Enrech (1983) found differences in corolla size between morphs in only one of three populations. Pailler and Thompson (1997) reported significant differences between LS and SS flowers for some floral dimensions among eight populations of *Gaertnera vaginata* (Rubiaceae). In some populations, LS flowers had larger petals or stigmas whereas in other populations the opposite pattern was documented. Heterostyly may thus vary among populations such that studies of single populations should not be taken to describe species. Such variation suggests that individual populations may be under different selective regimes associated with this sexual system or that these traits may not be under strong selection.

TABLE 5. Regression analysis of mean anther height per plant and mean stigma height per plant vs. mean corolla height per plant for long-styled (LS) and short-styled (SS) flowers in each population studied. The difference between the slopes of the regression lines for mean anther and stigma heights within floral morph (e.g., LS anther vs. LS stigma of *B. ternifolia* at the Santa Catalinas) was tested for each population of the heterostylous species. *$P < 0.05$, ***$P < 0.001$, ns = not significant, — = no test done. Population abbreviations: Santa Catalina Mountains, SCM; Santa Rita Mountains, SRM; La Selva, LAS; Soberanía National Park, SNP.

Species	Population	Morph	Floral organ	No. plants[a]	Slope[b]
B. ternifolia	SCM	LS	Anther	25	0.76
		LS	Sitgma	25	0.79 ns
		SS	Anther	25	0.99
		SS	Stigma	25	0.47***
B. ternifolia	SRM	LS	Anther	10	0.88
		LS	Stigma	10	1.03 ns
		SS	Anther	8	0.95
		SS	Stigma	8	1.13 ns
P. poeppigiana	LAS	LS	Anther	11	0.88
		LS	Stigma	11	0.27*
		SS	Anther	24	0.93
		SS	Stigma	24	0.5***
P. poeppigiana	SNP	LS	Anther	8	0.56
		LS	Stigma	8	0.66 ns
		SS	Anther	9	0.97
		SS	Stigma	9	0.81 ns
P. chiapensis	LAS	LS	Anther	11	0.43
		LS	Stigma	11	0.66 ns
		SS	Anther	10	0.82
		SS	Stigma	10	1.04 ns
P. brachiata	LAS	—	Anther	13	0.27
		—	Stigma	13	0.14 —
P. pittieri	LAS	—	Anther	11	0.49
		—	Stigma	11	0.29 —

[a] Number of plants from which flowers were taken.
[b] Slopes are significantly different from zero in all cases except stigma vs. corolla for *P. brachiata* and *P. pittieri*.

Among-species comparisons—Comparisons among heterostylous species reveal the range in expression of this sexual system and permit recognition of general vs. species-specific patterns. Differences in anther and stigma heights are most often compared among taxa but dimorphisms in corolla, stigma, pollen grain size, and pollen production have been found in a number of distylous taxa (Dulberger, 1992).

Ganders (1979) stated that dimorphism in corolla size was rare among heterostylous taxa and that, when found, the SS morph always had the larger corolla. This pattern was documented here for *Bouvardia ternifolia* and the two species of *Psychotria,* except that corolla length in the LAS population of *P. poeppigiana* did not differ between morphs. Differences in corolla length between LS and SS flowers have been recorded for a number of other heterostylous Rubiaceae. Short-styled flowers have larger corollas or corolla tubes in *Rudgea jasminoides* (Baker, 1956), *Palicourea fendleri* and *P. petiolaris* (Sobrevila, Ramírez, and de Enrech, 1983), *Luculia gratissima* (Murray, 1990), *Guettarda scabra* (Richards and Koptur, 1993), *Manettia luteo-rubra* (Passos and Sazima, 1995), *Hedyotis salzmannii* (Riveros, Barría, and Humaña, 1995), *Gaertnera vaginata* (Pailler and Thompson, 1997), and *Palicourea padifolia* (Ree, 1997). Among Rubiaceae studied, only in *Psychotria suerrensis* (Stone, 1995) are LS flowers larger than SS flowers. The relationships between floral morph and corolla size suggest that heterostyly may be under phylogenetic or ontogenetic constraints in Rubiaceae (but see Faivre, 2000).

We found that stigma size in SS flowers exceeded LS flowers in all three heterostylous species studied. Although Ganders (1979) and Dulberger (1992) have suggested that differences between LS and SS stigmas are not consistent, Rubiaceae that have been studied quantitatively show this same pattern. Two species of *Palicourea* (Sobrevila, Ramírez, and de Enrech, 1983), *Luculia gratissima* (Murray, 1990), *Hedyotis caerulea* (Ornduff, 1980), *Neanotis montholoni* (Bahadur, Laxmi, and Rama Swamy, 1984), *Palicourea lasiorrachis* (Feinsinger and Busby, 1987), and *P. padifolia* (Ree, 1997) have SS flowers with significantly larger stigmas than LS flowers. In some species, stigma shape is also variable. Descriptions and illustrations of flowers studied by Richards and Koptur (1993), Riveros, Barría, and Humaña (1995), and Pailler and Thompson (1997) suggest that LS flowers have wider, more capitate stigmas, whereas those of SS flowers are more elongate. This difference in stigma shape was observed here for *P. poeppigiana.* In terms of functional significance, Feinsinger and Busby (1987) and Ree (1997), working with different species of *Palicourea,* suggested that larger stigmas of SS flowers increased pollen receipt and thus female reproductive success of that floral morph.

Differences among taxa in these dimorphic characters have been related to phylogenetic constraints (Anderson, 1973; Lloyd and Webb, 1992a), morphological constraints (Ganders, 1979), genetic linkage (Lewis and Jones, 1992), and incompatibility system (Dulberger, 1992). The three species that we studied permit initial exploration of the roles of phylogenetic constraints and of pollinators in explaining differences in the expression of heterostyly among taxa.

Phylogenetic constraints—As described above, many heterostylous Rubiaceae show similar patterns of differences between SS and LS flowers. The distribution of heterostyly in the family indicates that it is unlikely that heterostyly has evolved only once in all taxa that share these characteristics (Faivre, Clauss, and McDade, unpublished data). Heterostyly thus has apparently evolved via parallel changes to yield a heterostylous syndrome that is characteristic of Rubiaceae. Contrary to expectations based on phylogenetic proximity, *B. ternifolia* and *P. chiapensis* were similar in having highly variable stigma and anther heights among flowers of the same morph (Figs. 3 and 5). Flowers of *P. poeppigiana,* in contrast, have stigmas and, to a lesser degree, anthers in distinct height classes corresponding to floral morph (Fig. 4). In fact, the two psychotrias were similar only in having a greater distance between stigma and anthers in LS flowers than in SS flowers; flowers of *B. ternifolia* showed no clear pattern. The expression of heterostyly in these species thus seems independent of phylogenetic relatedness. However, rigorous examination of the role of phylogeny will require well-resolved phylogenies onto which the evolution of traits involved in heterostyly can be optimized.

Pollinator relationships and the expression of heterostyly—Pollinators have a direct role in selection on floral morphology such that differences in pollinator relationships might explain variation in the expression of heterostyly. For example, in contrast to hummingbirds' bills, the proboscides of hawk moths are narrower and often inserted at an angle, with implications for the precision of pollen placement and thus degree of selective pressure on positioning of floral structures. However, flowers of the two hummingbird-pollinated species, *B. ternifolia* and *P. poeppigiana,* were not notably more similar in the expression of heterostyly than they were to flowers of the hawk moth-pollinated species, *P. chiapensis.*

It should be noted that flowers of *B. ternifolia* and *P. poeppigiana* were visited by different species of hummingbirds. Some experimental evidence suggests that hummingbirds of different species place pollen differently (Campbell, Waser, and Price, 1994). Further, whereas the corolla is the attractive organ in *B. ternifolia,* visitors to flowers of *P. poeppigiana* are presumably attracted by the large, red bracts subtending inflorescences; individual flowers are not brightly colored and are relatively small. This difference reinforces the point that the "hummingbird syndrome" is highly variable. Still, the flower is the unit of reproduction and must function in pollen receipt and donation.

Reciprocity—By definition, distylous taxa have flowers of two distinct morphologies with anthers and stigmas at reciprocal heights (Darwin, 1877; Barrett, 1990). Reciprocal anther and stigma heights are essential components in models explaining the evolution and maintenance of heterostyly based on enhanced outcrossing (Darwin, 1877; Charlesworth and Charlesworth, 1979b; Lloyd and Webb, 1992b). Assuming that pollinators are positioned in a similar manner when visiting LS and SS flowers, strictly reciprocal stigma and anther heights should ensure that pollen from one floral morph is deposited on the pollinator's body in a location that matches the position of the stigma of the opposite floral morph. If this is so, then the absolute value of the difference between anther and stigma heights should be the same in LS and SS flowers of the same species.

This expectation was upheld, in this study, for *B. ternifolia* (both populations; Table 1), but not for flowers of either species of *Psychotria* (Tables 2 and 3). Comparable data have only rarely been presented for other heterostylous species.

However, in three species of Rubiaceae, *Pentas lanceolata* (Bahadur, 1970), *Gaertnera vaginata* (Pailler and Thompson, 1997), and *Palicourea padifolia* (Ree, 1997), and in a species of *Gelsemium* (Loganiaceae) (Ornduff, 1970), there was greater separation between anthers and stigmas in LS than in SS flowers. For *Guettarda scabra* (Richards and Koptur, 1993) the opposite was recorded, with greater separation between stigmas and anthers of SS flowers. For *Manettia luteo-rubra* (Passos and Sazima, 1995), distances were not measured but graphs suggest no differences between floral morphs. Ree (1997) found significantly greater disassortive pollen flow in LS flowers vs. SS flowers of *Palicourea padifolia* and related this to proximity of anthers and stigma within SS flowers. He noted that the greater distance between stigma and anthers within LS flowers may reduce intramorph pollen flow compared with that found in SS flowers.

We extended our examination of reciprocity to incorporate the facts that these species have epipetalous stamens with only very short free filaments and that corollas vary in length. Regression analysis indicated that there is a relationship between corolla length and both anther height and stigma height in all populations of heterostylous species. This is noteworthy because only anthers are physically linked to the corolla. The fact that stigma height also varied with corolla length may be indicative of selection for distyly. To achieve reciprocity in stigma and anther heights, stigma height not only must change in concert with anther height as corolla length varies among individuals, but that change must maintain the same distance between stigma and anthers within flowers. That is, both within and between morphs, the slope of the relationship between corolla length and stigma height should be the same as that between corolla length and anther height.

Psychotria chiapensis, one population each of *P. poeppigiana* (SNP) and *B. ternifolia* (SRM), and LS flowers of *B. ternifolia* from SCM conform to this expectation. However, in SS flowers of *B. ternifolia* from SCM, different rates of change resulted in a greater distance between stigma and anthers in larger flowers. Hence, some plants of the SS morph had stigmas and anthers that were not reciprocal to those of LS flowers. For flowers of both morphs of *P. poeppigiana* at LAS, the distance between anthers and stigmas varied with corolla length. The impact of these deviations from reciprocity on mating systems depends upon the dynamics of pollen removal and deposition. If these processes are quite precise, there might be a significant nonrandom component to mating in the population. That is, if LS and SS plants with flowers of the same length have stigmas and anthers that are reciprocal to one another, then plants with like-sized corollas might exchange pollen more frequently than plants that differ in corolla length.

The plants we have studied deviate markedly from expectations for heterostylous species. Stigma and anther heights vary among individuals of the same morph and overlap between morphs in most populations. Further, these organs are not always positioned reciprocally between floral morphs. To understand the fitness consequences of the lack of strict reciprocity, floral morphology must be linked to pollinator behavior and pollen transfer. There may be enough imprecision in pollen transfer that deviations from reciprocity of the magnitude reported here have little impact on seed set. However, Ganders (1979) found that, for flowers of *Lithospermum californicum* (Boraginaceae), differences of as little as 2 mm in stigma and anther height in LS flowers significantly affected the level of legitimate pollination. This suggests that variation

among individuals of the magnitude documented here could certainly affect fitness.

Evolutionary gain or loss of heterostyly—When Richards and Koptur (1993) and Eckert and Barrett (1994) found variation in stigma and anther heights similar to that found in *B. ternifolia* and *P. chiapensis,* they suggested that evolutionary "breakdown" of heterostyly was underway. It is equally possible that the populations we studied are in the process of gaining heterostyly. In most cases in which the evolutionary status of heterostyly has been questioned, stigma and anther heights are not reciprocal and plants are also self-compatible (Riveros, Arroyo, and Humaña, 1987; Barrett, 1989; Negrón-Ortiz, 1996). Evolutionary transitions from heterostyly may be reflected not only by floral morphology and the incompatibility system, but also by morph frequencies within a population (Barrett and Richards, 1990; Barrett, Jesson, and Baker, 2000). Plants of the LS and SS floral morphs are expected to be present in a 1:1 ratio in heterostylous populations; skewed morph ratios may indicate the breakdown of heterostyly (Charlesworth and Charlesworth, 1979a). In the most extreme cases, one floral morph may become extinct in a population (Barrett, 1985; Owens et al., 1993). One of the most common hypotheses regarding factors responsible for the breakdown of heterostyly is loss of pollinators, especially in populations at the edge of the species' geographical (Richards and Koptur, 1993) or elevational range (Ornduff, 1975).

The plant populations studied here do not fit any of the situations described above in which breakdown of heterostyly is occurring. Pollinators were active, and fruits and seeds were produced in all populations. In all populations, plants with LS and SS flowers were present in approximately equal proportions (A. Faivre, unpublished data). Similarly, all plants that we studied had typical heterostylous incompatibility systems (Faivre, 1998), even the few plants of *B. ternifolia* (SCM) and one of *P. chiapensis* that consistently produced nearly homostylous flowers (i.e., with little separation between stigma and anthers) (A. Faivre, unpublished data). It seems unlikely that these aspects of heterostyly would be firmly established in populations in transition to or from this sexual system.

Approach herkogamous species—The AH floral morphology is widely interpreted as a mechanism for reducing self-pollination and enhancing outcrossing (Faegri and van der Pijl, 1979). Webb and Lloyd (1986) propose that herkogamy enhances pollinator precision and reduces self-interference (i.e., interference between pollen receipt and dispatch through floral architecture). Approach herkogamy has also been proposed as one of the ancestral conditions from which heterostyly has evolved (Lloyd and Webb, 1992b). The AH species of *Psychotria* were included in this study to provide a comparison of floral morphology in related plants that lack the heterostylous sexual system. Notably, whereas both anther and stigma height were related to corolla length in the heterostylous species, only anther height was so related in the AH species. This indicates that the covariation between anther and stigma height that is key to heterostyly is lacking in the AH species. Regarding variation in stigma and anther height, if plants with AH flowers are under selective pressure simply to ensure that stigma contact occurs prior to anther contact by pollinators (Webb and Lloyd, 1986), then AH flowers should show more variation in stigma and anthers heights than heterostylous flowers. Heterostylous plants, in comparison, must maintain

both reproductive organs at consistent heights relative to each other and among two or three floral morphs. In fact, flowers of *P. pittieri* are highly variable especially in stigma height (Fig. 6d) and the distance between stigma and anthers within a flower (note extremely high CVs for these variables [Table 4] compared to CVs for other species [Tables 1–3]). However, stigma and anther heights in *P. brachiata* are fairly consistent among individuals (Fig. 6a, b), and CVs for these floral dimensions are similar to those for the heterostylous species (Table 4).

Thus, flowers of *P. brachiata* are approach herkogamous: the distance between stigma and anther heights is consistent and varies little among plants. Flowers of *P. pittieri* show more variability in stigma heights with the result that flowers on some plants are AH, others homostylous, and a few are slightly reverse herkogamous (anthers exserted above the stigma). Preliminary data suggest that both *P. brachiata* and *P. pittieri* are self-compatible (A. Faivre, unpublished data). Webb and Lloyd (1986) propose that in self-compatible, AH plants, there should be stronger selection for maintenance of separation between stigma and anthers than in self-incompatible taxa. However, this does not seem to be the case for plants of *P. pittieri*. Similar variability in stigma and anther heights has been recorded for *Ipomopsis aggregata* (Polemoniaceae), but this AH species is self-incompatible (Campbell, Waser, and Price, 1994).

Studies of within-population variability in AH flowers are useful in evaluating the basic assumptions of Lloyd and Webb's (1992b) model for the evolution of heterostyly. One of the initial assumptions is that all flowers in a population are AH and that the presence of a reverse herkogamous flower would represent a new mutation. However, it appears that putatively AH taxa may include some plants with homostylous and others with reverse herkogamous flowers. Assuming that these characteristics are under genetic control, these may represent stages in the transition to heterostyly following the Lloyd and Webb (1992b) model. Such plants provide opportunities to test the model's predictions regarding relative fitness of different morphs.

Conclusions—We have documented deviation from the expectation of strict reciprocity between stigma and anther positions in flowers of three heterostylous species using three different approaches. First, stigma and anther heights (measured from the top of the inferior ovary) were compared among individuals across each population (Figs. 3–5). Second, the distance between stigma and anther heights within individual flowers was determined and compared within and between morphs (Tables 1–3). Finally, anther and stigma heights were related to variation in corolla length within and between morphs (Table 5). Notably, these different perspectives on heterostyly led to somewhat conflicting conclusions about degree of deviation from typical heterostyly among populations and species. Only *P. poeppigiana* had stigma and anther heights in two discreet classes. However, in terms of distance between stigma and anthers within flowers, *B. ternifolia* appeared most closely to meet expectations for distylous taxa (i.e., the mean distance between stigmas and anthers within flowers was not significantly different between floral morphs). In contrast, for both psychotrias the distance between stigma and anthers was greater in LS flowers. Finally, regression analyses suggested that variation in corolla length would result in differences in the degree of separation of anthers and stigmas among plants

of *B. ternifolia* (SCM) and *P. poeppigiana* (LAS). Clearly, the relative utility of these approaches to quantifying heterostyly and deviation therefrom requires further work. These and the reciprocity indices proposed by Lloyd, Webb, and Dulberger (1990), Richards and Koptur (1993), and Eckert and Barrett (1994) will be compared in a subsequent paper.

Both our work and the limited literature reporting results of population-level studies of floral morphology (Richards and Koptur, 1993; Eckert and Barrett, 1994; Pailler and Thompson, 1997) suggest that deviation from typical heterostyly, as documented here, is not especially uncommon. Still, additional studies are needed and it is critical that these report data on floral organs on an individual basis rather than solely population means. Heterostylous taxa should be sampled widely, including both monocots and dicots and, within dicots, both other asterids (with epipetalous stamens) and members of other subclasses. Such data will be key to understanding patterns of variation in the expression of heterostyly across angiosperms.

Finally, the deviation that we document from typical heterostyly begs the question of functionality. Not enough is known about the fitness consequences of floral morphological variation to permit evaluation of the variation documented here. Is the magnitude of deviation from strict reciprocity in these species within a functional range, or is it indeed likely to decrease significantly legitimate pollen transfer? Pollinator behavior and pollen transfer must be linked specifically to variation in floral morphology. We fully realize that such work is difficult and painstaking, but the evolutionary causes and consequences of heterostyly will not be clear until these functional aspects of heterostyly are understood.

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