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FLORAL VARIATION AND DISTYLY IN *GUETTARDA SCABRA* (RUBIACEAE)¹

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Controversy exists as to whether the tropical shrub *Guettarda scabra* (Rubiaceae) is distylous. Variations in stigma and anther position and floral morphology of *G. scabra* were studied in a population in south Florida. Stigma and anther height have unimodal distributions, but stigma-anther separation is bimodally distributed and can be used to identify a long-styled and a short-styled morph. Stigma width varies between morphs, but anther length, pollen diameter, and stigma papillae length do not. The morphs occur in a 1:1 ratio in the two populations studied. *G. scabra* is self-compatible and can pollinate itself. Styles of the two morphs have similar relative growth rates in early development. Styler growth is inhibited in the short-styled morph when buds are approximately 12 mm long. Anther height differs between morphs because of different relative growth rates and because the long-styled morph corolla tube, where the anthers are attached, stops growth before the tube of the short-styled morph. Reciprocity between morphs for average stigma and anther height falls within the range of reciprocity found in other distylous Rubiaceae. Thus *G. scabra* is morphologically distylous but unusual among distylous species in the variation within morphs and overlap between morphs in stigma and anther heights.

Since heterostyly was first recognized by Hildebrand and Darwin, the primary character used to define the syndrome is the presence of a reciprocal stigma-anther height polymorphism (Darwin, 1877; Ganders, 1979a; Lloyd, Webb, and Dulberger, 1990; Lloyd and Webb, 1992a). This condition is described as reciprocal herkogamy, because the stigmas and anthers are spatially separated within a flower (herkogamy) and are reciprocally positioned between floral morphs (Webb and Lloyd, 1986; Lloyd and Webb, 1992a). In distylous species the long-styled (L) morph has long styles and short stamens, while the short-styled (S) morph has short styles and long stamens that match the level of the opposite type of reproductive organ in the L morph. Experimental evidence indicates that reciprocal herkogamy promotes pollination between morphs in heterostylous species (Ganders, 1974; Barrett and Glover, 1985; Lloyd and Webb, 1992b). Many heterostylous species also have self- and intramorph incompatibility systems, as well as ancillary floral polymorphisms, such as pollen size, pollen number, and stigma shape differences (Dulberger, 1992).

Although reciprocal herkogamy of anthers and stigmas is the basic feature that defines heterostyly and is necessary in order to promote intermorph pollination, species are often described as heterostylous based solely on the observation of inverse herkogamous flowers, without quantitative data establishing stigma-anther reciprocity. Some species, such as *Mirabilis froebelii* and *Epacris impressa*, lack heterostyly but have inverse herkogamous flowers in populations (Baker, 1964; O'Brien and Calder, 1989; stilar polymorphisms are reviewed in Barrett and Richards,

1990, and in Barrett, 1992). Quantitative morphological data are necessary in order to compare positions of stigmas and anthers among flowers and to recognize morphs in a population. Such data are especially important in investigations of species like *Guettarda scabra*, where the arrangement of stigmas and anthers appears to differ from that of typical heterostylous species.

G. scabra (Rubiaceae) is a shrub of pinelands and subtropical hardwood forests (hammocks) and has a circum-Caribbean distribution. Species of *Guettarda* have been described as distylous (Bahadur, 1968; Zapata and Arroyo, 1978), as polymorphic but not heterostylous (Tomlinson, 1974), and as lacking distyly (Verdcourt, 1958; Robbrecht, 1988). Styles in *Guettarda* species are either exerted or included within the floral tube, while anthers are subsessile and inserted at the throat of the tube (Bacigalupo, 1957; Tomlinson, 1974, 1980). Upon casual inspection, therefore, anther height does not appear to vary reciprocally with style length, because anther position is always the same. If corolla tube length varied inversely with stigma height, however, then anther height would vary similarly, and *Guettarda* species could be distylous.

This study investigated floral variation and compatibility relations in *G. scabra* in order to determine whether it is distylous and to establish how it compares to other distylous species. In addition, the developmental basis for differences between floral morphs was studied in order to examine hypotheses on the evolution of distyly.

MATERIALS AND METHODS

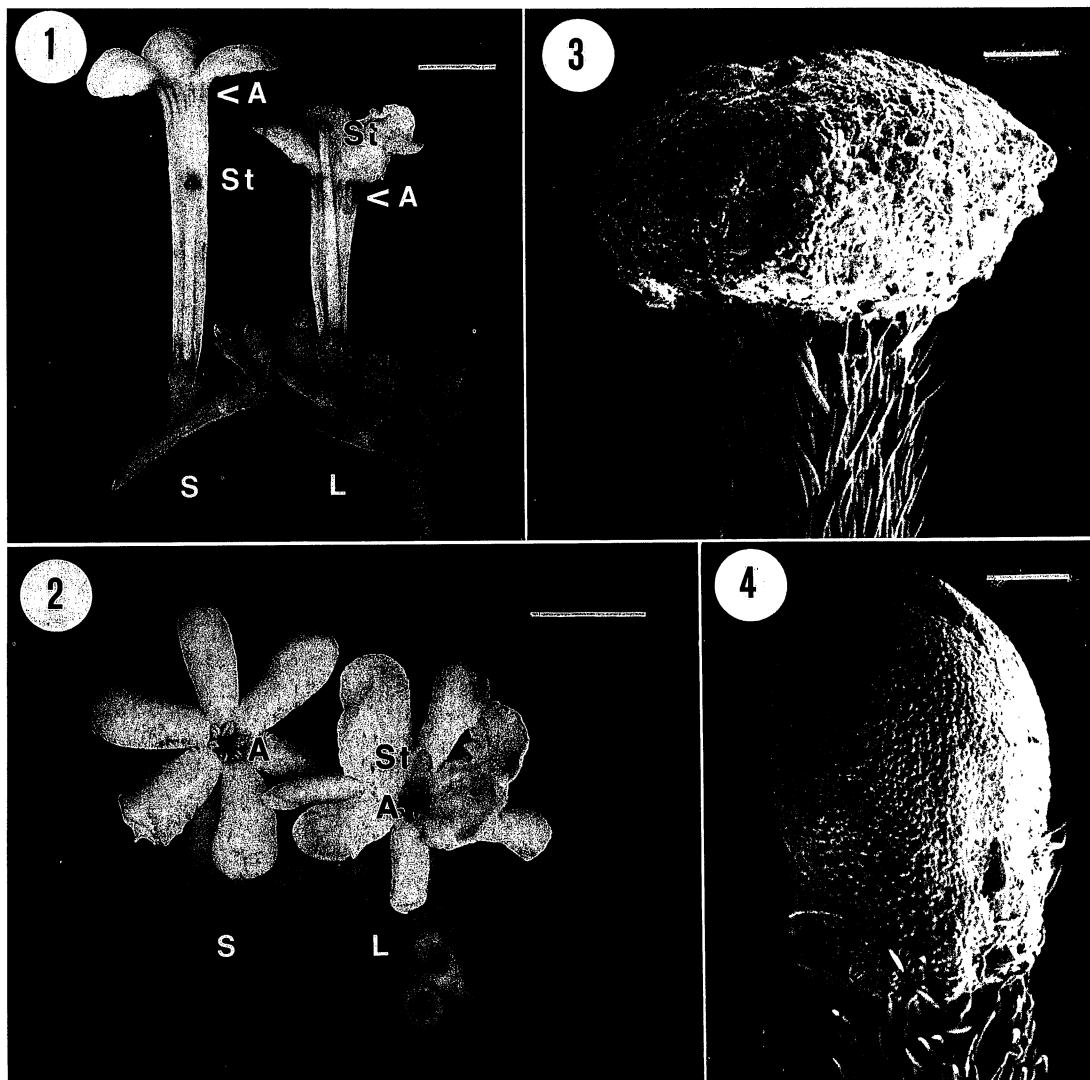
Plants of *Guettarda scabra* L. were studied in a pine-lands population adjacent to Redd Hammock, Long Pine Key, Everglades National Park, Florida, from 1986 to 1989. Morphs were also censused in a population at Navy Wells Park in Dade County, Florida, in 1991.

Population floral variation—Floral measurements were made on fresh material or material fixed in chromic acid-acetic acid-formalin (CRAF III; Berlyn and Miksche,

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Figs. 1–4. *Guettarda scabra*. L = long-style morph; S = short-styled morph; A = anthers; St = stigma. 1. Cymose inflorescence branches of L and S morphs bearing erect buds and flowers that have been cut longitudinally to expose styles and anthers. Bar = 5 mm. 2. Corolla throat of intact L and S flowers. Anthers are visible at throat of both morphs, while stigma is exerted from L morph flower. Bar = 5 mm. 3. SEM micrograph of stigma of L morph. Bar = 0.2 mm. 4. SEM micrograph of stigma of S morph. Bar = 0.2 mm.

1976). Corolla tube length, anther height, and stigma height were measured from the top of the inferior ovary. Pollen from mature but undehiscent anthers was used to determine pollen grain diameter. Stigma papillae cell length was measured on stigmas softened in 5 M NaOH, then stained with 0.05% toluidine blue O in phosphate buffer (pH 7.0). Measurements were made with a ruler, a dissecting microscope, or a compound light microscope equipped with either a calibrated ocular micrometer or a video image analysis system. Morph ratios were compared using a *G* statistic (Sokal and Rohlf, 1981). Student's *t*-test or a nested analysis of variance (ANOVA) was used to analyze flower measurements. Sample sizes are given in tables.

In order to compare frequency distributions of organ lengths in *G. scabra* to variation in a distylous shrub from the same family, stigma and anther height in flowers of *Psychotria nervosa* (Tomlinson, 1974; Burch, Wunderlin, and Ward, 1975), which grew in the same habitat as *G. scabra*, were similarly measured and analyzed.

Floral variation within plants—Ten randomly selected plants of *G. scabra* were marked prior to flowering, then all open flowers on each plant were collected at weekly intervals. Floral variation within and between individuals was compared with a one-way ANOVA.

Developmental studies—Buds and mature flowers from ten plants of each morph were fixed in formalin-acetic acid-alcohol (Berlyn and Miksche, 1976). Organ lengths of these buds were measured, then anthers were stained with 0.05% toluidine blue O in distilled water, squashed, and examined to assess pollen developmental stage. Flower growth rates were determined in the field through compilation of daily, every other day, or weekly measurements made on a total of 15 plants. Data were transformed to the natural logarithm (\ln) and fitted with least squares regression lines. Slopes of lines were compared using Student's *t*-test. When slopes were not significantly different, an analysis of covariance (ANCOVA) was used to compare intercepts.

Compatibility studies—Compatibility studies were performed on 65 marked individuals over two flowering seasons in May–July 1986 and 1987. The first flowers to open on an inflorescence were used for hand-pollinations. On each inflorescence all open flowers (usually one, sometimes two) were pollinated at time of treatment. Inflorescences were enclosed in small organza bags on the day prior to anthesis of the first flower. The inflorescence remained bagged during fruit development in order to prevent pollination of the other flowers on the inflorescence. The four pollination treatments were autogamy (= automatic self-pollination), hand self-pollination, intramorph pollination, and intermorph pollination. In the autogamy treatment flowers were not hand-pollinated but were bagged as in the other treatments. In 1986 individual flowers pollinated were not marked and emasculations were not performed. In 1987 an additional set of flowers was emasculated by removing the corolla below the anthers prior to anthesis, then bagged, in order to test for nonpseudogamous apomixis.

Fruit set was recorded as positive if at least one fruit was set per inflorescence. In all treatments fruit set was recorded after fruits were fully developed, approximately 1–2 months postpollination. Fruit set was compared with Fisher's exact test.

RESULTS

Guettarda scabra flowers from May until August in southern Florida. Flowers are borne on long-stalked axillary dichasia that produce three to 19 flowers. Flowers open in the late afternoon around 1630 to 1730 EST and emit a strong, sweet odor. On the morning after anthesis the corolla lobes begin to brown, and most flowers abscise by midday. Flowers are radially symmetrical with a 1- to 2-mm bilobed calyx and salverform corolla (Fig. 1). Numbers and dimensions of floral parts are given in Table 1. Anthers are subsessile and attached at the mouth of the corolla tube (Figs. 1, 2). Anther number usually equals corolla lobe number and ranges from 3 to 9, with an average of 6 (Table 1). Because anthers are always located at the corolla mouth, anther height varies with corolla tube length. The single style, which emerges from a nectariferous disc at the top of the ovary, has a papillate stigma that varies in shape from cylindrical to capitate (Figs. 3, 4).

Population floral variation—Stigma and anther heights have continuous distributions in a population of *G. scabra* (Fig. 5A, B). Stigma-anther separation, however, is bimodal (Fig. 5C). These distributions contrast with those of *Psychotria nervosa*, a distylous shrub in the Rubiaceae that also occurs at Redd Hammock. Stigma and anther heights of *P. nervosa* are bimodal, even though there is some overlap in the distribution of heights between morphs, while stigma-anther separation divides the plants into two distinct morphs (Fig. 6).

Stigma-anther separation was used to divide the *G. scabra* population into L and S morphs. Plants that had stigmas and anthers at the same level (i.e., 0 stigma-anther separation on Fig. 5C) were grouped with L plants. Stigma and anther heights of these two groups of plants have different distributions (Fig. 7A, B), and mean stigma and

TABLE 1. Measurements of floral parameters for *Guettarda scabra* from Redd Hammock, Everglades National Park, Florida. Data are mean \pm standard deviation

	Long-styled plants	Short-styled plants	P-value
Corolla tube length (mm) ^a	15.1 \pm 2.9	18.6 \pm 3.2	<0.001
Corolla lobe length (mm) ^a	6.1 \pm 0.9	6.0 \pm 0.8	NS ^b
Number of corolla lobes ^a	6.1 \pm 0.8	6.2 \pm 1.0	NS
Number of anthers ^a	6.0 \pm 1.2	6.2 \pm 1.0	NS
Anther length ^c	2.6 \pm 0.3	2.5 \pm 0.3	NS
Pollen diameter (μ m) ^d	36 \pm 4	37 \pm 3	NS
Anther height (mm) ^a	14.0 \pm 2.5	17.6 \pm 3.1	<0.001
Stigma height (mm) ^a	16.9 \pm 3.3	13.5 \pm 2.2	<0.001
Stigma length (mm) ^a	1.0 \pm 0.2	1.0 \pm 0.2	NS
Stigma width (mm) ^a	1.3 \pm 0.2	1.0 \pm 0.2	<0.001
Papillae cell length (μ m) ^e	53 \pm 11	49 \pm 8	NS
Ovary locule number ^f	4.0 \pm 0.8	4.6 \pm 0.7	NS
Stigma-anther separation (mm) ^a	2.0 \pm 2.0	-4.1 \pm 0.7	<0.001

^a $N = 38$ for L plants and $N = 53$ for S plants.

^b NS = $P > 0.05$.

^c $N = 40$ anthers for L plants and 49 anthers for S plants; one anther/plant.

^d $N = 50$ pollen grains/plant measured for five plants/morph.

^e $N = 10$ plants/morph; 30 cells/plant measured.

^f $N = 10$ L and 10 S plants; three flowers/plant counted.

anther heights differ significantly (anther height, $F = 143.58$ [$P < 0.0001$]; stigma height, $F = 266.92$ [$P < 0.0001$]). The unimodal distributions of these characters in the population (Fig. 5A, B) result from overlap in stigma and anther height between the two groups. The overlap is more extensive for anther height than for stigma height (Fig. 7). In the ANOVA for stigma height, between-group variance accounts for 42% of the total variation, whereas between-group variance comprises 28% of total variation in anther height. Average stigma height in the L morph roughly corresponds to average anther height in the S morph and vice versa (Table 1).

Among the other floral characters measured, only stigma width differs significantly between morphs (Table 1; Figs. 3, 4). Short-styled plants tend to have stigmas that are narrow and cylindrical, while stigmas on L plants are generally capitate. As with stigma and anther height, however, the distribution of stigma widths overlaps between morphs. The papillae of stigmas in both morphs are composed of chains of secretory cells, but terminal cell length in these chains does not differ significantly between morphs (Table 1).

The ratio of L plants to S plants was 1:0.96 (190:182; $N = 372$) in the Redd Hammock population and 1:1.05 (61:64; $N = 125$) at Navy Wells. A G -test shows that these ratios do not differ significantly from 1:1 (Redd Hammock: $G = 0.172$; $P = 0.678$; Navy Wells: $G = 0.072$; $P = 0.788$). A G -test for heterogeneity indicates significant variation among the three samples at Redd Hammock ($N_1 = 72:44$; $N_2 = 48:58$; $N_3 = 70:80$; $G_H = 8.266$; $P = 0.016$), but not at Navy Wells ($N_1 = 32:30$; $N_2 = 29:34$; $G_H = 0.390$; $P = 0.532$).

Floral variation within plants—Although stigma and anther height vary among plants in a population, stigma height, anther height, and stigma-anther separation are relatively uniform on single plants over time. One-way ANOVAs comparing variation in stigma height, anther

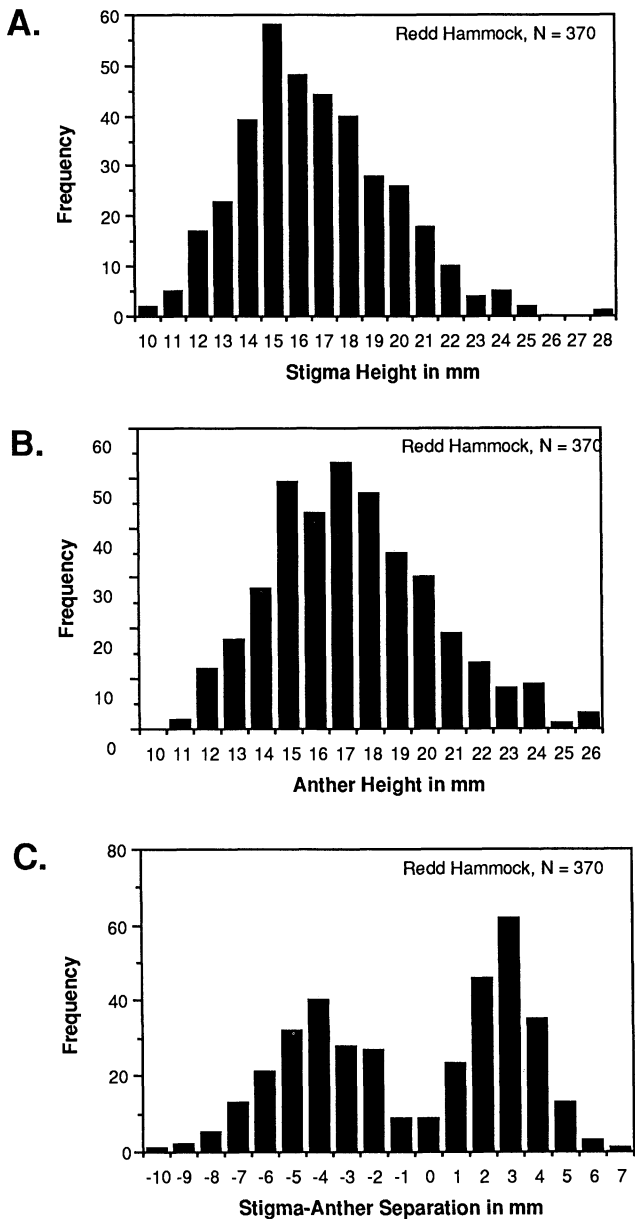


Fig. 5. Distribution of stigma height (A), anther height (B), and stigma-anther separation (C) for flowers of *G. scabra* from the Redd Hammock population.

height, and stigma-anther separation among flowers collected from ten plants throughout their flowering season revealed significant differences among plants for these three parameters ($P < 0.0001$). Differences among individuals accounted for 73.7% of the variation in stigma height and 85.3% of the variation in anther height. A comparison of variation in corolla lobe length for the same sample showed no significant differences among individuals ($P = 0.9395$), and all of the variation in lobe length was accounted for by variation within individuals.

Single plants have less variation in stigma-anther separation than the total range of variation in a morph (Fig. 8). Flowers that have stigmas and anthers at the same height may represent the extremes of floral variation in plants such as Individual 5, Fig. 8, rather than homosty-

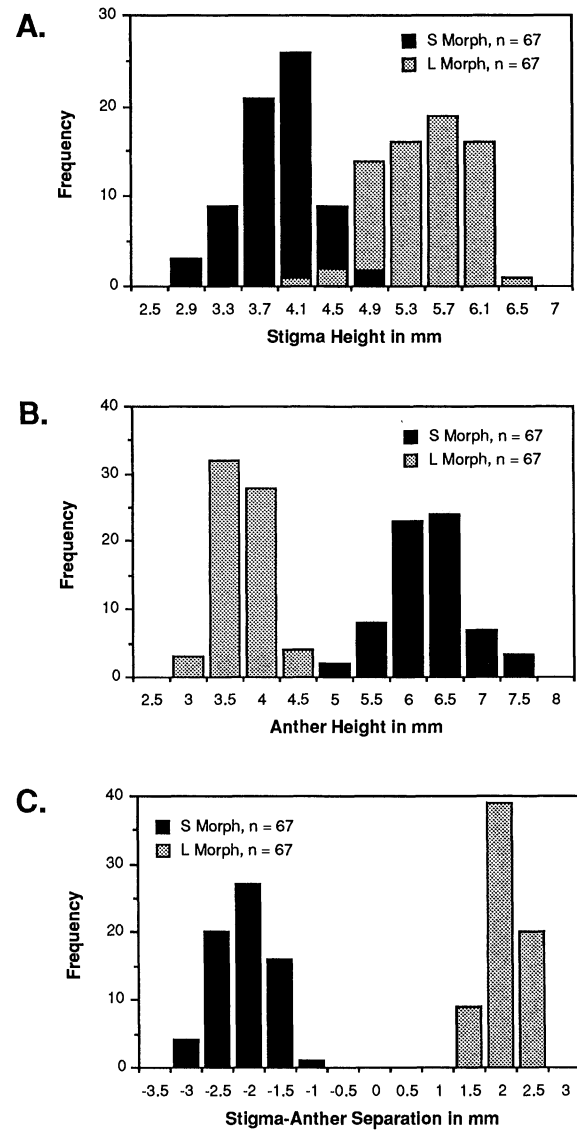


Fig. 6. Distribution of stigma height (A), anther height (B), and stigma-anther separation for flowers of *Psychotria nervosa* from Redd Hammock.

lous plants. The ten plants sampled did not change style morph during the growing season and had relatively uniform stigma-anther separation throughout the flowering season (Fig. 9). No plant switched style morph in 91 plants monitored for two seasons.

Development of differences in stigma and anther heights

Stigma height differs between the two morphs because of a late-developing difference in relative growth rates (Fig. 10A). The styles of the L and S morphs have similar relative growth rates in young buds. In buds ca. 12 mm (ln 9.4 μm) in length, however, stylar growth is inhibited in the S morph, while the L morph continues along the original developmental trajectory (Fig. 10A). Regressions of ln bud length vs. ln stigma height have similar slopes for buds less than 12 mm long, but slopes are significantly different for larger buds (Table 2). An analysis of covariance for buds less than 12 mm long shows a significant

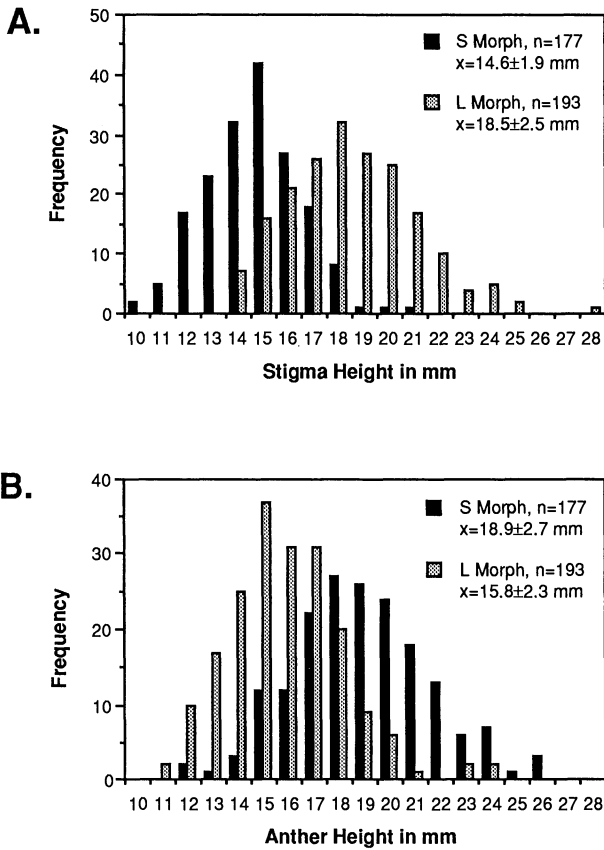


Fig. 7. Distribution of stigma height (A) and anther height (B) for data in Fig. 5 after the population was divided into morphs based on stigma-anther separation.

difference between intercepts for the two morphs, but calculation of these differences indicates that the actual difference in style length at 300 μm bud length, which was used as an approximation of bud size at gynoecium initiation, is less than 3 μm . This early difference therefore cannot account for the mature differences in stigma height.

Measurements of bud growth rate in the field, when correlated with relative growth data from dissected buds, showed that buds take ca. 3 weeks to develop from meiosis to anthesis. The difference in style length appears during the last 2 days of growth, as the buds begin to elongate rapidly.

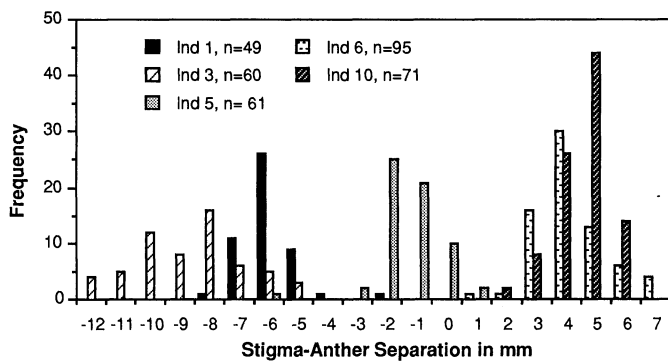


Fig. 8. Distribution of stigma-anther separation for flowers on five individuals from the Redd Hammock population. For each plant, flowers were collected at weekly intervals throughout the flowering season.

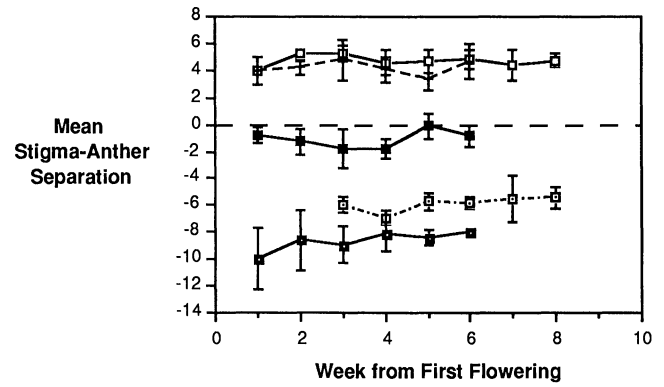


Fig. 9. Average stigma-anther separation over time for flowers from five plants in the Redd Hammock population. Error bars represent two standard deviations.

Relative growth of \ln bud length vs. \ln anther height is linear in both the L and S morphs (Fig. 10B; Table 2). When regression lines for \ln bud length vs. \ln anther height are compared, however, the slopes of the L and S morphs are significantly different, even for buds less than 12 mm in length (Table 2). In order to eliminate the autocorrelation resulting from the dependence of anther height on corolla tube length, \ln anther height in the two morphs was also regressed against \ln stigma height for buds less

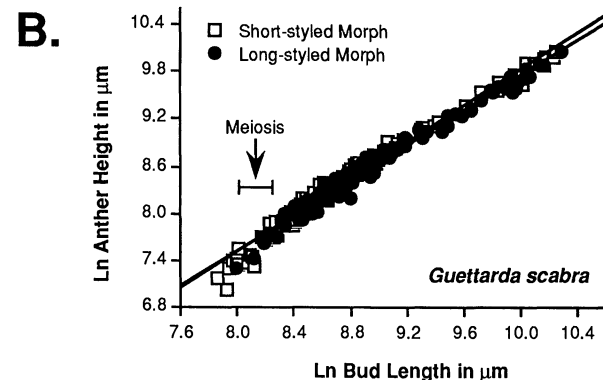
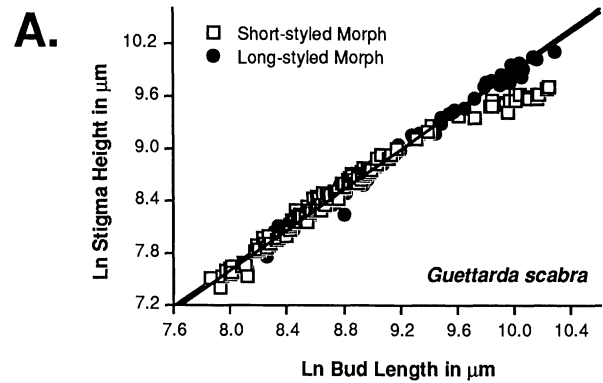


Fig. 10. Relative growth rate of \ln bud length vs. \ln stigma height (A) and \ln bud length vs. \ln anther height (B) for L and S morphs, ten individuals per morph, from the Redd Hammock population.

TABLE 2. Regression equations for floral developmental parameters in *Guettarda scabra*. Slopes are given + twice their standard errors, which approximates their 95% confidence intervals. *T*-values are for a comparison of the difference between the two slopes immediately above

	Slope	Y-intercept	R ²	N
Ln Bud L × Ln Stigma Ht				
Buds ≤ 12 mm				
L Morph	1.166 ± 0.038	-1.729	0.972	108
S Morph	1.176 ± 0.030	-1.795	0.982	108
	<i>t</i> = -0.419, <i>P</i> = 0.676			
Buds > 12 mm				
L Morph	1.079 ± 0.092	-0.922	0.954	28
S Morph	0.540 ± 0.072	4.151	0.917	22
	<i>t</i> = -8.962, <i>P</i> = 0.000			
Ln Bud L × Ln Anther Ht				
L Morph	1.108 ± 0.026	-1.338	0.982	136
S Morph	1.167 ± 0.028	-1.837	0.981	130
	<i>t</i> = -3.013, <i>P</i> = 0.003			
Buds ≤ 12 mm				
L Morph	1.234 ± 0.048	-2.432	0.961	108
S Morph	1.311 ± 0.046	-3.064	0.970	108
	<i>t</i> = -2.310, <i>P</i> = 0.022			
Ln Stigma Ht × Ln Anther Ht				
Buds ≤ 12 mm				
L Morph	1.056 ± 0.026	-0.579	0.984	108
S Morph	1.117 ± 0.021	-1.079	0.991	108
	<i>t</i> = -3.634, <i>P</i> = 0.000			

than 12 mm in length (Table 2). Significant differences between morphs were found in this latter comparison. Relative growth rate of ln anther height is greater in the S morph. In addition, the L morph flowers mature at smaller bud lengths than S morph flowers (Table 1).

Compatibility studies—When flowers were emasculated and bagged, virtually no fruit was set (0% S morph, *N* = 32 flowers; 3% L morph, *N* = 28 flowers), which shows that *G. scabra* lacks nonpseudogamous apomixis. Fifty-one percent of the inflorescences studied, however, set fruit autogamously (Table 3), indicating that both morphs are self-compatible and that substantial fruit set can take place in the absence of pollinators. Because we did not

initially emasculate and monitor individual flowers, some of the fruit set on inflorescences in 1986 cross-pollinations may be autogamous. Nonetheless, when the results for inflorescences with self-pollinations and intramorph crosses (illegitimate pollinations) are compared to those for intermorph crosses (legitimate pollinations), inflorescences with legitimate pollinations showed 23% greater fruit set over inflorescences with illegitimate pollinations (Table 3). This difference is significant (*P* = 0.033).

DISCUSSION

Our results show that *G. scabra* is morphologically distylous because populations have two morphs with reciprocal stigma and anther heights. Other aspects of the heterostyly syndrome are exhibited in the 1:1 morph ratios and the ancillary polymorphism in stigma width. This species is atypical, however, in its degree of floral variability, its compatibility relationships, and its corolla polymorphism.

Although *G. scabra* is morphologically distylous, we do not know whether its heterostyly promotes disassortative pollinations between morphs, i.e., whether its distyly is functional. Heterostyly may promote intermorph pollinations in *G. scabra*, in which case the floral variability is merely an extreme example of the variability accommodated in heterostylous systems. Reports of stilar variation in *G. uruguensis* (Bacigalupo, 1957) and *G. elliptica* (S. Koptur, unpublished data) indicate that stilar variability may be common in the genus, rather than confined to *G. scabra*. Alternatively, morphological heterostyly in *G. scabra* could be a relic of a more conventional heterostyly that has broken down under pressures such as pollinator uncertainty at the edge of its range. In this case, the species would be primarily self-fertilizing and the morphological variation would be nonfunctional. Breakdown frequently occurs in heterostylous species (Charlesworth and Charlesworth, 1979; Ganders, 1979a; Barrett, 1988) and might be favored in south Florida, where *G. scabra* is at the northern limit of its range. Under this hypothesis, *G. scabra*'s variable stigma and anther heights result from relaxation of the developmental canalization that maintains reciprocal herkogamy in a distylous species, while mean stigma and anther height reflect the ancestral distyly. Clearly, additional data on floral variation in the genus,

TABLE 3. Results of field hand-pollination experiments on *Guettarda scabra*

Morph	Pollination treatment	N	% Fruit set	Legitimate vs. illegitimate crosses ^a
Short	Autogamy	45	44.4	<i>P</i> = 0.193
	Self-pollination	43	67.4	
	Legitimate cross	28	78.6	
	Illegitimate cross	96	67.1	
Long	Autogamy	27	63.0	<i>P</i> = 0.040*
	Self-pollination	27	59.3	
	Legitimate cross	36	77.8	
	Illegitimate cross	44	56.8	
Morphs combined	Autogamy	72	51.4	<i>P</i> = 0.033*
	Self-Pollination	70	64.3	
	Legitimate	64	78.1	
	Illegitimate	140	64.3	

^a Probabilities from one-tailed Fisher's exact test, *df* = 1, * = significant (*P* < 0.05).

along with detailed studies of gene flow within the species, are needed to distinguish between these hypotheses and to understand the evolutionary and ecological implications of floral variation in *G. scabra*.

Floral variability—Published distributions of stigma and anther heights in distylous species usually show two relatively discrete morphs (Martin, 1965; Lewis and Rao, 1971; Opler, Baker, and Frankie, 1975; Barrett, 1978; Barrett and Richards, 1990), as seen in the data presented here for *Psychotria nervosa*. Anther height overlaps significantly, however, in some species (Dulberger, 1973; Opler, Baker, and Frankie, 1975; Ganders, 1979b). Two of these latter species, *Linum pubescens* and *Erythroxylum coca*, have two stamen series per flower, and the overlap occurs between the longer stamen series of the L morph and the shorter series of the S morph. Species with intermorph overlap in anther heights show complete or almost complete separation of style lengths between morphs.

G. scabra differs from these distylous species in having unimodal distributions of style length and anther height. In addition, when stigma-anther separation is used to define two morphs, a large amount of overlap in organ length occurs between morphs in both anther and stigma height. Since *Guettarda* has only a single stamen series, differences in height between anther series cannot account for the overlap in anther height between the morphs.

Anchusa hybrida, *A. officinalis*, *Narcissus tazetta*, and *Quinchamalium chilense* have unusual stylar heteromorphisms (Barrett and Richards, 1990). Individuals of these species have flowers either with stigmas below anthers or with stigmas at or above the anthers, but all lack stigma-anther reciprocity. Stamen and style lengths in the two morphs of *Q. chilense* have separate distributions, based on published means and ranges (Riveros, Arroyo, and Humana, 1987). The three other species show either continuous distributions of style and anther heights (*A. officinalis*; Philipp and Schou, 1981) or bimodal distribution of style lengths, accompanied by overlap between morphs in anther length (*A. hybrida*; Dulberger, 1970) or by no difference between morphs in anther level (*N. tazetta*; Dulberger, 1964). *G. scabra* differs from these species with stylar polymorphisms, because it has an average anther height dimorphism that varies reciprocally with the average stigma height dimorphism. Because *G. scabra* has reciprocal stigma and anther height dimorphisms, this species is more clearly distylous than the species described above, and the biological effect of its floral variability should be considered in the context of our understanding of distyly.

Compatibility—Heterostylous species usually display self- and intramorph incompatibility and lack mechanisms for self-pollination. Typical heterostylous incompatibility has been found in other distylous Rubiaceae (Bawa and Beach, 1983; Sobrevila, Ramirez, and de Enrech, 1983; Murray, 1990), although self-compatibility has also been reported (Bawa and Beach, 1983; Sobrevila, Ramirez, and de Enrech, 1983). Compatibility relations in *G. scabra* are atypical for distylous species because the species is both self- and intramorph compatible and capable of pollinating itself (autogamy). Because the flowers

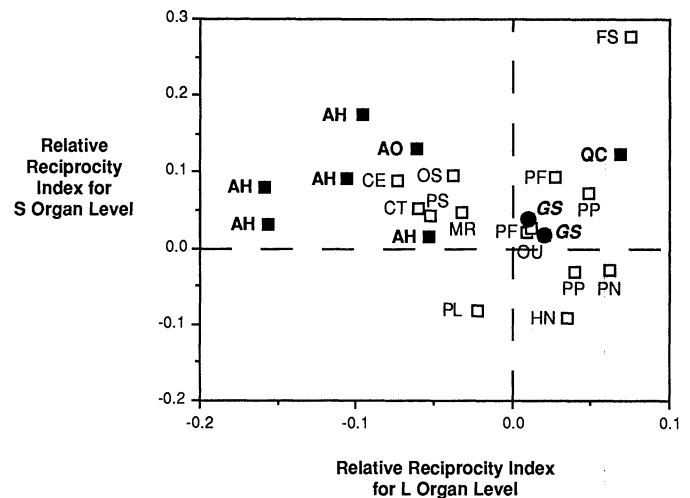


Fig. 11. Relative reciprocity ratio for the long organ level vs. the short organ level for distylous species in the Rubiaceae and species with stylar polymorphisms. Abbreviations for species are adjacent to each point on the graph. Abbreviations with references in parentheses are: AH = *Anchusa hybrida* (Dulberger, 1970); AO = *Anchusa officinalis* (Philipp and Schou, 1981); CE = *Cephaelis elata* (J. H. Richards, unpublished data); FS = *Fareamea suerrensis* (J. H. Richards, unpublished data); GS = *Guettarda scabra*; HN = *Hedyotis nigricans* (Bahadur, 1970a); MR = *Mitchella repens* (Ganders, 1975); OU = *Oldenlandia umbellata* (Bahadur, 1963); OS = *Oldenlandia scopulorum* (Bahadur, 1966); PF = *Palicourea fendleri* (Sobrevila, Ramirez, and de Enrech, 1983); PL = *Pentas lanceolata* (Bahadur, 1970b); PP = *Palicourea petiolaris* (Sobrevila, Ramirez, and de Enrech, 1983); PN = *Psychotria nervosa*; PS = *Psychotria suerrensis* (J. H. Richards, unpublished data); QC = *Quinchamalium chilense* (Riveros, Arroyo, and Humana, 1987). Species with unusual stylar heteromorphisms are denoted with solid squares. *G. scabra* is marked with solid circles. All other species are rubiaceae species that have been reported to be distylous. Data from more than one sample are included where available.

are erect, autogamy in the S morph could result from pollen being shed onto the S stigma. How autogamy occurs in the L morph is less apparent. Pollen could germinate and penetrate the side of the style, as has been found in *Spigelia* (Loganiaceae) (K. Grove, personal communication), or pollen might be shed in the bud prior to stylar elongation, as happens in many protandrous Rubiaceae (Robbrecht, 1988). In our dissections of flower buds, however, we saw no evidence of precocious pollen shedding. Alternatively, selfing could occur when corollas abscise, as has been demonstrated for *Mimulus guttatus* (Dole, 1990).

Despite the potential for autogamy, morphs in *G. scabra* occur in a 1:1 ratio in the populations studied. Cryptic self-incompatibility and embryo abortion or inbreeding depression have been hypothesized to preserve 1:1 morph ratios in other self-compatible, distylous species (Weller and Ornduff, 1977, 1989, 1991; Casper, 1985; Casper, Sayigh, and Lee, 1988). Our studies show that legitimate pollinations result in greater fruit set than illegitimate pollinations in *G. scabra*, indicating that the species may have a weak self-incompatibility system. Studies of pollen tube growth, embryo development and seed set, as well as genetic analyses of paternity, are needed in order to determine whether there are differences among pollination treatments in seed set and to distinguish between

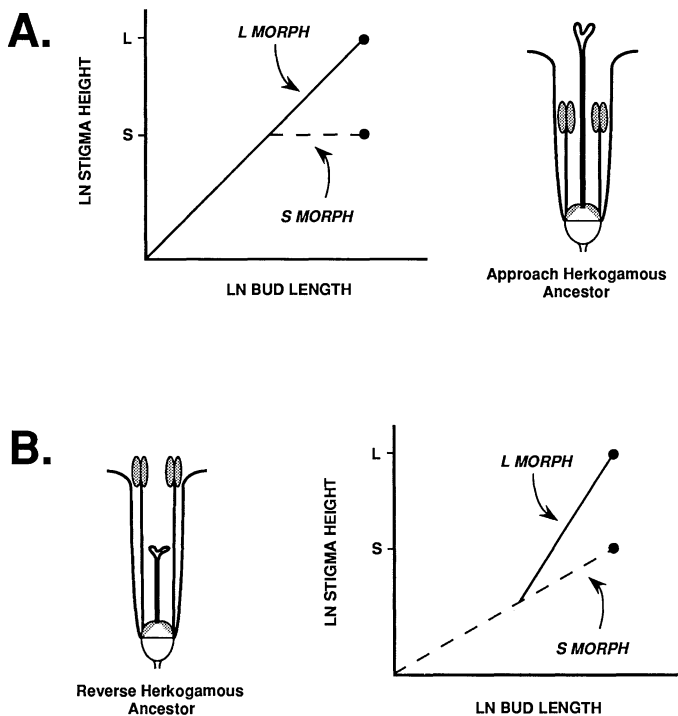


Fig. 12. Hypothetical graphs for relative growth of bud length vs. stigma height in a distylous species with an approach herkogamous ancestor (A) or a reverse herkogamous ancestor (B). (A) Initial relative growth rate leads to the L morph; the S morph develops by an inhibition or deceleration of the initial rate. (B) Initial relative growth rate leads to the S morph; the L morph develops by an acceleration of the initial rate.

prezygotic and postzygotic factors contributing to maintenance of the 1:1 morph ratio.

Reciprocal herkogamy—Although the amount of variation in stigma and anther height is unusual in *G. scabra*, when stigma-anther separation is used to separate the population into two morphs, the morphs have roughly reciprocal mean stigma and anther heights. J. H. Richards, D. G. Lloyd, and S. C. H. Barrett (unpublished data) have derived several indices that quantify reciprocal herkogamy and allow comparison of the degree of average herkogamy and reciprocity among heterostylous species. One of these indices measures the relative reciprocity between organ levels and is calculated for each organ level as $(\text{Anther Height} - \text{Reciprocal Stigma Height}) / (\text{Anther Height} + \text{Reciprocal Stigma Height})$. In species that are perfectly reciprocal the index equals zero for both organ levels. Figure 11 shows the relative reciprocity index for the long organ level vs. short organ level in reportedly distylous rubiaceaceous species and species with unusual stylar polymorphisms. This figure shows that the indices for *G. scabra* are not particularly unusual and are closer to zero than other distylous Rubiaceae. *A. hybrida*, *A. officinalis*, and *Q. chilense*, in contrast, differ substantially from zero. Thus, the average lengths of reproductive organs in *G. scabra* are reciprocally herkogamous, and the reciprocity falls within the range found in distylous species in the Rubiaceae.

Developmental basis—A late-developing divergence in growth rates is primarily responsible for the observed differences in style length in *G. scabra*. Theoretically, either morph could show the late developmental divergence, because the initial relative growth rate could produce a long style (Fig. 12A), a short style (Fig. 12B), or final growth rates of both the long and short styles could diverge from the initial rate. Styler development in *G. scabra* follows a model similar to Fig. 12A. Lloyd and Webb (1992a) have argued that distyly evolves most frequently in species with “approach herkogamous” flowers, which have styles exerted beyond the anthers. The developmental pattern seen in *G. scabra* is consistent with the hypothesis that distyly evolved in an ancestor with an exerted style (e.g., Fig. 12A) and that the S morph arose via a mutation that interfered with stylar elongation.

In contrast to stylar dimorphism, differences in anther height in *G. scabra* can be attributed to at least two sources. First, stamens in the S morph have a greater relative growth rate than those in the L morph. Because stamens are attached on the throat of the corolla tube, this difference may represent alternative modes of relative growth in length vs. breadth for corollas of the two morphs. The differences in growth rate, however, are slight and would result in only a 2-mm difference in stamen height for flowers 25 mm long, when the actual difference in stamen height is approximately 3–4 mm. The additional difference between morphs in anther height arises from differences in extent of corolla growth.

In *G. scabra* the anther height polymorphism, therefore, has a different and more complex developmental basis than the stigma height polymorphism. Although models similar to those presented in Fig. 12 can be generated for development of anther height polymorphisms in an approach herkogamous ancestor, the data presented here show that *G. scabra* morphs do not share a common pathway in early stamen development. Patterns for the development of heterostylous anther height differences, in general, are more varied than patterns for the development of stigma height polymorphisms (Richards and Barrett, 1987, 1992; J. H. Richards, unpublished data). These varied developmental patterns could reflect the inherently more complex developmental environment of anthers, which are temporally and positionally intercalated between petals and carpels.

The early divergence in stamen development seen in *G. scabra* is not found in other Rubiaceae examined to date (J. H. Richards, unpublished data). *Guettarda* is a well-defined taxon within the Rubiaceae (Robbrecht, 1988; Bremer and Jansen, 1991). Since other distylous Rubiaceae vary both position of filament insertion and filament length between morphs (Richards and Barrett, 1992), the invariance of these characters in *G. scabra* may reflect a restriction on filament development in this genus that has forced heterocorollary to be selected. Heterocorollary has also been reported in *Cordia sebestena* (Percival, 1974; Tomlinson, 1980), and quantitative studies may show that this species, like *G. scabra*, is distylous. Comparison of heterocorollous distylous species to distylous species with stamen length and position variations may provide clues to the structural or developmental factors that favor selection of variation in corolla tube length over filament length and position.

In summary, although anther position on the corolla tube does not vary in *G. scabra*, our quantitative data show that the species is morphologically distylous, because corolla tube length varies reciprocally between morphs. Other species in the Rubiaceae that have “incomplete heterostyly” (Verdcourt, 1958) may also be found to have reciprocal herkogamy when stigma and anther heights are measured. This study of floral variation in *G. scabra* illustrates the importance of a quantitative approach to defining heterostyly.

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