

## Heterostyly in the Lamiaceae: The case of *Salvia brandegeei*

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**Abstract.** Heterostyly rarely occurs in families with strongly zygomorphic flowers. For this reason Darwin (1877) doubted whether heterostyly would occur in the Lamiaceae and recent reviews have not reported the floral polymorphism in this family. Here we describe distyly in a rare species of *Salvia* restricted to bluffs and seaward canyons on Santa Rosa Island (Santa Barbara Co., California) and northwestern Baja California (Mexico). *Salvia brandegeei* is morphologically distylous with populations composed of equal frequencies of long- and short-styled morphs differing reciprocally in stigma and anther position. Controlled hand pollinations demonstrated no significant differences in the seed set of self, intramorph or intermorph pollinations. Unlike most heterostylous species investigated, *S. brandegeei* does not possess a diallelic incompatibility or ancillary polymorphisms of pollen and stigmas. We propose that the evolution of distyly in *S. brandegeei* may have been associated with an ecological shift to a new environment in which protandry failed to prevent increased levels of geitonogamy. Heterostyly was then selected because it increased the proficiency of cross-pollination. The origin of distyly in self-compatible *S. brandegeei* is consistent with Lloyd and Webb's theoretical model for the evolution of distyly.

**Key words:** Heterostyly, distyly, zygomorphic flowers, self-compatibility, protandry, geitonogamy, Lamiaceae, rare plant species.

### Introduction

Heterostyly is a genetic polymorphism characterized by the reciprocal placement of stigmas and anthers in flowers of animal-pollinated angiosperms (Darwin 1877, Barrett 1992a). Two classes of heterostyly occur depending on whether there are two (distyly) or three (tristyly) floral morphs within plant populations. Associated with the reciprocal arrangement of sex organs is usually a diallelic incompatibility system that prevents self and intramorph matings and differences between the floral morphs in the size and structure of pollen grains and stigmatic papillae (Ganders 1979, Dulberger 1992, Barrett and Cruzan 1994, Richards 1997). Because of the close association of these morphological and physiological traits heterostyly is generally regarded as a floral syndrome in which the component parts function in a co-ordinated manner to promote animal-mediated cross-pollination. Heterostyly has been documented in at least 28 angiosperm families most of which are unrelated, implying multiple independent origins of the syndrome.

Darwin (1877, p. 259) first pointed out that heterostyly does not occur randomly among animal-pollinated angiosperm families and is generally absent from those possessing strongly zygomorphic flowers.

"Plants which are already well adapted by the structure of their flowers for cross-fertilisation by the aid of insects often possess an irregular corolla, which has been modeled in relation to their visits; and it would have been of little or no use to such plants to have become heterostyled. We can thus understand why it is that not a single species is heterostyled in such great families as the Leguminosae, Labiatae, Scrophulariaceae, Orchideae, &c., all of which have irregular flowers."

More recent work on the phylogenetic distribution of heterostyly has confirmed Darwin's observations. Heterostyly is generally absent from specialized families with strongly zygomorphic flowers and occurs more often in those with simple, actinomorphic, or occasionally weakly zygomorphic flowers, with open corollas and well developed floral tubes (Ganders 1979, Barrett 1992b, Dulberger 1992, Lloyd and Webb 1992a). Such flowers have been described as depth-probed and stereomorphic because pollinators probe for nectar without any particular bilateral orientation (Leppik 1972, Lloyd and Webb 1992a).

The Lamiaceae has often been specifically identified as a family in which heterostyly is unlikely to evolve because of the strongly zygomorphic flowers of many of its members. Indeed modern reviews on the distribution of heterostyly have not included any species from this family (e.g. Vuilleumier 1967, Ganders 1979, Barrett and Richards 1990, Barrett 1992b, Lloyd and Webb 1992a) and a recent review of breeding systems in the Lamiaceae did not mention heterostyly (Owens and Ubera-Jiménez 1992). However, several overlooked papers report heterostyly in members of the large genus *Salvia*. Haque and Ghoshal (1981) reported "heterostylic and homostylic" flowers in several *Salvia* species; however, in some cases individual plants exhibited mixed flower types, casting doubt on their interpretation of this floral variation and whether it involves a true genetic polymorphism. More significantly, Epling (1940) reported floral dimorphism in *Salvia brandegeei* Epling, a rare species from southern California and north-

west Baja California, and Neisess (1984) subsequently reported distyly for this taxon in a short note illustrating two floral morphs. Since no quantitative data on floral variation were presented in these papers, and because of the potential evolutionary significance of the occurrence of heterostyly in the Lamiaceae, we decided to investigate *S. brandegeei* to determine the nature of floral dimorphism and whether it possesses features typical of the heterostylous syndrome.

### Materials and methods

*Salvia brandegeei* is a shrub with small pale blue to lavender zygomorphic flowers in section *Audibertia* of the genus. This section contains 18 species all of which are confined to arid portions of the southwest USA (Epling 1938). *Salvia brandegeei* is restricted to bluffs and seaward canyons in coastal scrub, occurring only on Santa Rosa Island (Santa Barbara County, California) and a 40 km strip of northwestern Baja California (Mexico) coastline from Punta Santo Tomas south to Punta Cabras (Neisess 1984). All material that we investigated experimentally originated from a random bulk seed collection collected in 1993 from a population at Cherry Canyon, Santa Rosa Island. Although the number of maternal parents in the sample was not recorded the population was easily divided into two roughly equal classes of plants with long-versus short-styles. To quantify floral morph ratios we sampled all plants in six populations on Santa Rosa Island and determined by *G*-tests whether floral morphs occurred at equal frequency, as is typical of many distylous species. All experimental work was performed on plants derived from field collections. Plants were grown under glasshouse conditions at 15–25 °C in 20 cm clay pots containing sandy loam soil on a single bench at the University of Toronto. After several years of vegetative growth and only sporadic flowering, plants bloomed prolifically in 1998 and in a synchronous manner enabling us to measure floral traits and initiate a crossing program to determine the compatibility status of floral morphs.

We obtained floral measurements using a dissecting microscope and digital calipers. Only fresh flowers with mature sex organs in which styles were fully mature (see below) were sampled. For each flower we measured the following traits from

the base of the ovary: flower length, stigma position, anther position and length, stamen insertion position and staminode insertion position. Sample sizes are provided in the appropriate tables. We also measured the length and width of a total of 80 pollen grains produced by the long- and short-level anthers of four short- and five long-styled morphs, respectively, to determine whether like many heterostylous plants they differed in size. Comparisons of floral traits were made using ANOVA with flower size as a covariate. All ANOVAs in this study used JMP (version 3.0.2; SAS Institute 1994).

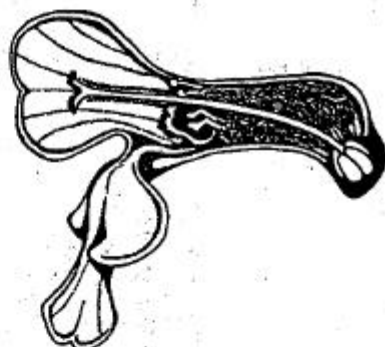
Flowers of *S. brandegeei* last for 4–5 days depending on temperature or whether they have been pollinated and, like many mints, are protandrous. We quantified floral longevity and temporal aspects of protandry by following a cohort of 36 unpollinated flowers throughout their anthesis period. To determine the compatibility status of each morph we performed three classes of controlled pollinations using fine forceps on multiple flowers on four long-styled and six short-styled plants. Flowers were either selfed, crossed with pollen from a plant of the same floral morph (intramorph) or crossed with a plant of the alternate floral morph (intermorph). Pollinations were only performed on flowers that had entered female function as indicated by the bifurcation of the two stigma lobes. Following controlled pollinations, the type of cross was recorded using diagnostic paint spots at the base of the flower, and when fruits were mature (approximately three weeks) we counted the number of seeds produced by each pollination treatment. We used ANOVA to examine whether there were significant differences in seed set among the three pollination treatments.

To investigate whether there were morph-specific differences in female fertility in *S. brandegeei* we measured seed set resulting from natural pollination (hereafter open pollination). We sampled 10 fruits on 4–5 plants of each morph in two natural populations on Santa Rosa Island (South-east Anchorage & Lower Cherry Canyon) and in two experimental garden trials at Santa Barbara Botanic Gardens in 1997 and 1999. We examined sources of variation in seed set by ANOVA. The model included site, morph, site  $\times$  morph interaction and plants nested within morph and site (all effects random except morph).

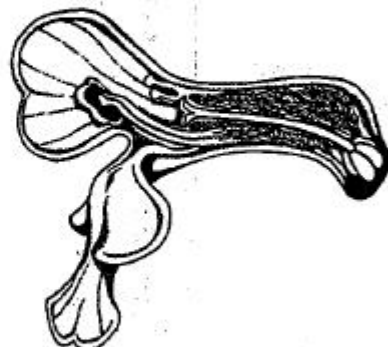
## Results

**Floral dimorphism.** Observations of natural populations of *S. brandegeei* on Santa Rosa Island revealed the presence of two distinct floral morphs differing in style length (Fig. 1). There was no significant deviation from 1:1 morph ratios among the six natural populations ( $G_{\text{pooled}} = 0.15$ ,  $df = 1$ , NS;  $G_{\text{het}} = 2.05$ ,  $df = 1$ , NS; Table 1). Measurements of the relative positions of stigmas and anthers in flowers from plants growing under uniform glasshouse conditions at Toronto confirmed that the species is distylous. There was a clear bimodal distribution of style length in the sample of flowers measured (Fig. 2) with long-styled plants possessing short-level anthers and short-styled plants with long-level

Long-styled morph



Short-styled morph



4 mm

Fig. 1. Flowers of the long- and short-styled morphs of distylous *Salvia brandegeei* (Lamiaceae)

Table 1. Floral morph ratios in natural populations of *Salvia brandegeei* on Santa Rosa Island, California

Site	L-Morph	S-Morph	G test
Lower Cherry Canyon	38	42	0.20 ns
Upper Cherry Canyon	36	41	0.32 ns
Mouth of Windmill Canyon	21	17	0.42 ns
Jolla Vieja Canyon	49	56	0.47 ns
Cañada Lobo	57	49	0.60 ns
Southeast Anchorage	68	73	0.18 ns

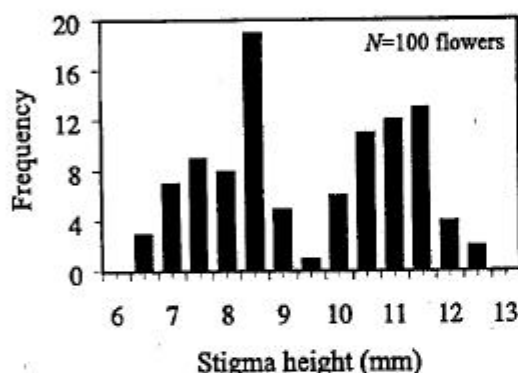


Fig. 2. The frequency distribution of stigma height (measured from the base of the ovary) in a sample of *Salvia brandegeei* flowers ( $N = 100$ ) from plants grown under uniform glasshouse conditions. See methods for details

anthers (Table 2, Fig. 3). Although sex-organ position was positively correlated with flower length (ANCOVA, Table 2), two discrete floral morphs, differing reciprocally in stigma and anther position, were evident (Fig. 3). Differences in anther position between the floral morphs largely reflect differences in filament length and their location on the upper portion of the floral tube (Fig. 1, Table 2). Both floral morphs possess staminodes but they are inserted at different positions on the upper part of the floral tube (Fig. 1, Table 2).

**Ancillary polymorphisms.** Our observations of *S. brandegeei* indicated that unlike

most heterostylous plants examined there are no ancillary polymorphisms of pollen or stigmas. Measurements of pollen grains from the long- and short-styled morphs indicated that they did not differ markedly in size. ANOVA on pollen length and width with morph (fixed effect) and flower nested within morph (random effect) indicated small but significant variation between flowers (length  $F = 6.75$ ,  $df = 6$ ,  $P < 0.0001$ ; width  $F = 3.21$ ,  $df = 6$ ,  $P = 0.0054$ ) but no significant morph effect (pollen length L-morph  $\bar{X} = 44.02 \mu\text{m}$ , S-morph  $\bar{X} = 43.87$ ,  $F = 0.03$ ,  $df = 1$ ,  $P = 0.862$ ; pollen width L-morph  $\bar{X} = 34.90 \mu\text{m}$ , S-morph  $\bar{X} = 33.82$ ,  $F = 1.55$ ,  $df = 1$ ,  $P = 0.241$ ). There were no significant differences in the size or structure of stigmas of the two floral morphs (width of cells on stigmatic surface were similar, L-morph  $\bar{X} = 11.25 \mu\text{m}$ ,  $N = 154$ ; S-morph  $\bar{X} = 11.31 \mu\text{m}$ ,  $N = 185$ ). There was also no significant difference in anther length (mm) between the style morphs (anther length L-morph  $\bar{X} = 18.9$ , S-morph  $\bar{X} = 13.2$ , ANCOVA {flower length, morph} model NS  $F = 2.92$ ,  $df = 2$ ,  $P = 0.06$ ). Based on material of *S. brandegeei* originating from Punta Cabras, Baja California, Neisess (1984) also reported that there were no differences in the mean volume of pollen grains (data not given) or in exine sculpturing between pollen grains from the two floral morphs.

**Protandry.** Observations of the longevity of unpollinated flowers of *S. brandegeei* in glasshouse-grown plants indicated that their average lifespan was  $\bar{X} = 4.75$  d with male function preceding female function (protandry). Average durations in the male and female phases were estimated at  $\bar{X} = 2.19$  d and  $\bar{X} = 2.56$  d, respectively, as judged by anther dehiscence and whether stigma lobes were reflexed.

**Compatibility relations.** Controlled hand pollinations of the long- and short-styled flowers of *S. brandegeei* under pollinator-free glasshouse conditions demonstrated that the species is fully self and intramorph compatible. There were no significant differences in the



Table 2. Mean position (mm) of floral traits in the long- and short-styled morphs of *Salvia brandegeei*. *F* values are from an ANCOVA with floral morph (fixed effect), plants within morph (random effect) and flower length (covariate). Measurements were made on 5 long-styled plants and 6 short-styled plants

Floral trait	Mean (SE)	<i>N</i> flowers	Morph		Plant (morph)		Flower length'	
			<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
	L-morph							
	S-morph							
Stigma position	11.01 (0.10)	50	440.14	<0.0001	1.49	0.180	62.86	<0.0001
	8.00 (0.10)	50						
Anther position	6.85 (0.09)	50	288.67	<0.0001	5.30	0.001	142.28	<0.0001
	11.27 (0.11)	50						
Stamen insertion position	7.03 (0.09)	50	37.02	<0.0001	1.24	0.290	82.89	<0.0001
	8.76 (0.18)	50						
Staminode position	7.59 (0.10)	20	23.31	0.0002	1.40	0.255	10.37	0.0029
	9.70 (0.18)	20						
Staminode insertion position	7.32 (0.21)	20	12.16	0.0066	3.05	0.030	7.63	0.009
	8.72 (0.27)	20						

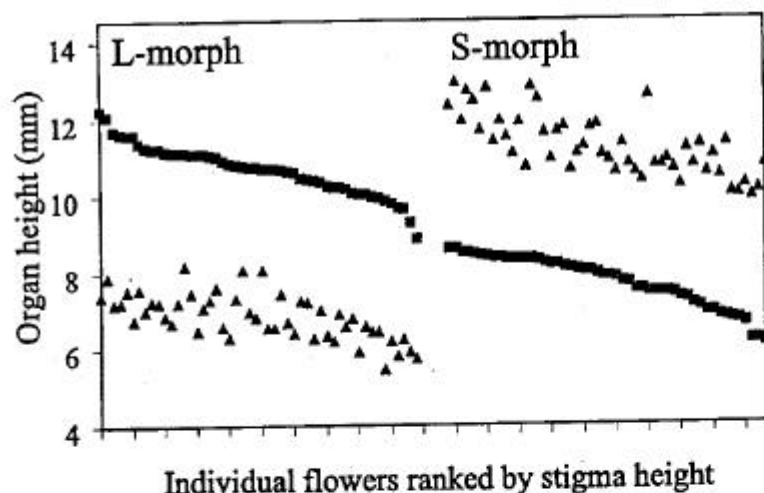


Fig. 3. Flowers of *Salvia brandegeei* ranked by style length to illustrate the reciprocal correspondence of stigma and anther positions in the long- and short-styled morphs. Positions of stigmas are indicated by squares (■) and those of anthers by triangles (▲)

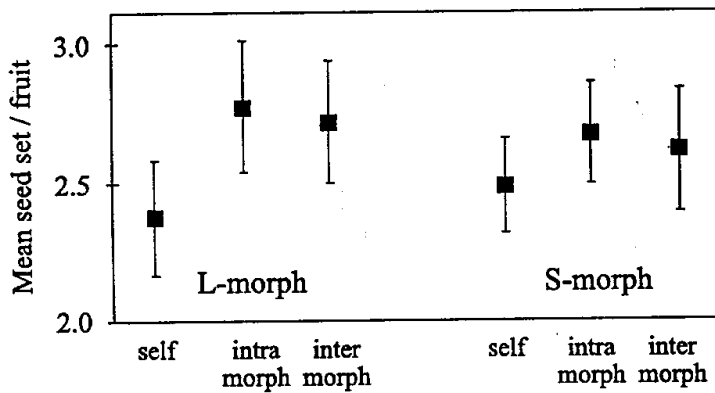
female fertility (seed set) of self versus outcross pollinations or between intramorph versus intermorph cross pollinations (Table 3; Fig. 4; fruit set  $F = 0.420$ ,  $P = 0.834$ ; seeds per fruit  $F = 0.487$ ,  $P = 0.486$ , ANOVA included morph, pollination treatment {self, intramorph, intermorph} and their interaction). Neisess (1984) also reported self-compatibility in plants of *S. brandegeei* from Punta Cabras. Collectively these results involving both mainland and island populations

of *S. brandegeei* clearly indicate that this species does not possess a diallelic self-incompatibility system typical of most heterostylous plants that have been examined.

**Natural seed set.** Comparisons of open pollinated seed set among natural and garden populations revealed no significant difference overall between the floral morphs or sites (grand mean: long-styled morph  $\bar{X} = 2.08$ , short-styled morph  $\bar{X} = 1.96$ ; ANOVA model NS,  $F = 0.918$ ,  $P = 0.964$   $df = 37,342$ ).

**Table 3.** Fruit and seed set following controlled hand pollinations of flowers of the long- and short-styled morphs of *Salvia brandegeei*

Floral morph	Treatment	N	Percent fruit set	Mean seed set/pollination	Mean seed set/fruit	(SE)	N fruit
L-morph	self	36	0.67	1.58	2.38	0.207	24
	intramorph	37	0.59	1.65	2.77	0.237	22
	intermorph	56	0.57	1.55	2.72	0.221	32
S-morph	self	70	0.61	1.53	2.49	0.171	43
	intramorph	64	0.53	1.42	2.68	0.183	34
	intermorph	46	0.57	1.48	2.62	0.222	26

**Fig. 4.** Mean seeds per fruit ( $\pm 1$  SE) following controlled hand pollinations of flowers of the long- and short-styled morphs of *Salvia brandegeei*. Plants of each morph were selfed, outcrossed to plants of the same morph (intramorph), and outcrossed to plants of the opposite morph (intermorph)

Pollinators observed visiting garden plants included mostly feral honey bees (*Apis mellifera*), but also bumblebees (*Bombus* spp.) and leaf-cutter bees (Megachilidae).

### Discussion

The unexpected discovery of an apparently single isolated origin of distyly in the large family Lamiaceae (approx. 5600 spp.) requires explanation. While in some well known heterostylous families hundreds of species possess the polymorphism (e.g. Boraginaceae, Oxalidaceae, Primulaceae, Rubiaceae) curiously heterostyly is represented by only one or a handful of species within genera of several other large families [e.g. Acanthaceae – *Oplonia* (Ornduff 1979a); Amaryllidaceae – *Narcissus* (Barrett et al. 1997); Hypericaceae – *Hypericum* (Ornduff 1979b); Iridaceae – *Nivenia*

(Goldblatt and Bernhardt 1990); Polemoniaceae – *Gilia* (Cochrane and Day 1994), Saxifragaceae – *Jepsonia* (Ornduff 1971)]. These isolated occurrences suggest that there may be strong constraints on the evolution of the polymorphism in these groups either because floral characters are inappropriate (see Lloyd and Webb 1992a) and/or the ecological conditions necessary for the selection of heterostyly occur rather infrequently. The fact that the only known case of distyly in the Lamiaceae is *S. brandegeei*, a rare species restricted to a few localities is particularly intriguing. *Salvia* is large (approximately 900 species, Mabberley 1987) and widespread in distribution and has likely confronted diverse pollination conditions during its evolutionary history. What particular features of *S. brandegeei* and the environments it occupies may have favoured the evolution of heterostyly is unclear.

Most *Salvia* species that have been investigated are self-compatible, cross-pollinated by animals (primarily bees), and exhibit protandrous, zygomorphic flowers (Huck 1992, Owens and Ubera-Jiménez 1992, Proctor, Yeo and Lack 1996, Navarro 1997). It seems reasonable to assume given the features of *S. brandegeei* that its immediate ancestor probably also possessed these characteristics. Assuming this to be true, one plausible evolutionary scenario that might explain the origin of heterostyly involves an ecological shift to a new environment in which the quantity or quality of pollinator service compared to that normally experienced by ancestral populations differed. In a new pollination environment protandry may have been less effective in reducing the mating costs associated with self-pollination (although see Harder et al. 2000). *Salvia brandegeei* can produce large floral displays (up to 30 flowers daily in glasshouse-grown plants) and our observations indicate that plants are often in both male and female phase simultaneously with flowers not ordered by position in any particular developmental sequence at the plant level (see Lloyd and Webb 1986). With inferior pollination (*sensu* Harder and Barrett 1996) resulting in higher levels of geitonogamy, heterostyly may have been selected in the new environment because it increased the proficiency of cross-pollination resulting in higher fertility and/or better quality offspring.

Interestingly another polymorphic sexual system, gynodioecy, is widespread in the Lamiaceae, occurring commonly in *Salvia* (Darwin 1877, Owens and Ubera-Jiménez 1992, Van Treuren et al. 1993). Ganders (1978, 1979) proposed that similar selective forces involving altered pollination environments and their effects on mating could be involved in the evolutionary origins of these two polymorphisms. However, why heterostyly rather than gynodioecy should have evolved in *S. brandegeei* in response to these conditions is not obvious. Information on the pollination biology and mating of this species may provide clues in resolving this problem.

Anderson (1973) proposed that protandry might often favour the evolution of distyly with the short-styled morph originating in ancestral long-styled populations through retardation of normal style elongation (and see Richards and Koptur 1993). Once stigma-height dimorphism establishes selection for anther-height dimorphism may then follow because it increases the proficiency of cross-pollination (Lloyd and Webb 1992a,b; although see Baker et al. 2000). In *S. brandegeei*, the absence of a well developed heterostylous syndrome, involving diallelic incompatibility or ancillary pollen and stigma polymorphisms, makes this simple developmental hypothesis to account for the evolution of distyly attractive. However, Anderson (1973) developed this idea in an effort to explain the widespread occurrence of both protandry and distyly in the Rubiaceae. If protandry does favour the evolution of distyly in some groups then it still remains a perplexing question why distyly is so rare in the Lamiaceae, especially since protandry is also common in this family.

The occurrence of distyly without diallelic self-incompatibility in *S. brandegeei* adds to the growing number of cases in which heterostyly is reported in self-compatible taxa (reviewed in Barrett and Cruzan 1994). Given the isolated occurrence of self-compatible distyly in *Salvia* it seems highly improbable that any species in this genus (or family) has ever possessed diallelic incompatibility. Indeed, there is still no convincing evidence for the occurrence of any form of physiological self-incompatibility in the Lamiaceae (Owens and Ubera-Jiménez 1992) and it therefore seems reasonable to postulate that morphological distyly in *S. brandegeei* evolved independently of self-incompatibility. This pattern is in accord with Lloyd and Webb's model (1992a, b) of the evolution of distyly. However, it is inconsistent with earlier theories that assumed diallelic incompatibility was a necessary precondition for the evolution of the stigma-anther height dimorphism that characterizes heterostylous plants (reviewed in Charlesworth

and Charlesworth 1979, Ganders 1979, Barrett 1992b).

Lloyd and Webb (1992a, b) proposed that the reciprocal arrangement of stigmas and anthers in heterostylous plants develops in populations to increase the mating proficiency of plants as male parents and that this evolutionary step is independent of whether the ancestral population is self-compatible or possesses diallelic incompatibility. In some heterostylous groups diallelic incompatibility may subsequently evolve to reduce selfing and inbreeding depression whereas in others, such as *S. brandegeei*, it may not. Neisess (1984) found garden mixtures of long- and short-styled plants of *S. brandegeei* set seed, whereas when plants were segregated by morph they did not, despite strong self-compatibility. This suggests that in *S. brandegeei* floral morphology alone may serve to discourage selfing and promote cross-pollination thus limiting any requirement for the evolution of physiological self-incompatibility. Indeed the occurrence of equal morph ratios in populations suggests that the mating system of this species is based on patterns of symmetrical disassortative (intermorph) pollen transfer. However, before the role of physiology can be ruled out as a mechanism promoting intermorph mating it will be necessary to determine if *S. brandegeei* possesses a cryptic self-incompatibility system. Marker gene studies of several other apparently self-compatible heterostylous plants have indicated a role for cryptic incompatibility in promoting disassortative mating (reviewed in Barrett and Cruzan 1994).

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