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FUNCTIONAL DIOECISM IN THE MALPIGHIACEAE: THE BREEDING SYSTEM OF SPACHEA MEMBRANACEA CUATR.[1]

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ABSTRACT

The breeding system of *Spachea membranacea* (Malpighiaceae), an understory tropical rain forest tree, was studied on Barro Colorado Island in central Panama. Two types of trees were found, those with hermaphroditic flowers and those with female flowers. Crossing experiments indicated that morphological hermaphrodites normally function as males but may be induced to set fruit if emasculated prior to cross-pollination. Thus, although morphologically gynodioecious, *S. membranacea* is functionally dioecious. This is the first experimental evidence for functional dioecism in the Malpighiaceae. The proportions of hermaphrodites (functional males) in the three populations examined were 44.7% ($N = 47$), 60.0% ($N = 10$), and 87.5% ($N = 17$). The distribution of hermaphrodites and females in size classes was quite similar, suggesting that sexual expression is not influenced by the age of the individual. Flowers are visited and presumably pollinated primarily by bees of the genera *Paratetrapedia* (Anthophoridae) and *Trigona* (Apidae).

RECENT breeding system surveys in tropical plant communities have shown that dioecism is common among tropical trees (Bawa and Opler, 1975; Ruiz and Arroyo, 1978; Bawa, 1980; Sobrevila and Arroyo, 1982). This realization has prompted a closer examination of plant breeding systems in general and consideration of the selective factors involved in the evolution of dioecism in particular (Bawa and Beach, 1981; Baker, 1984; Charlesworth, 1984). Unfortunately, a plant's functional breeding system is not always accurately reflected by its floral morphology, a situation that is particularly evident in some species whose hermaphroditic flowers are functionally male (Arroyo and Raven, 1975; G. Anderson, 1979; Atsatt and Rundel, 1982). Thus, it is important to evaluate sexual expression on a functional rather than morphological basis as has been emphasized by Lloyd (1980), Ross (1982), and Charlesworth (1984).

W. R. Anderson (1977, 1981) was the first to note the existence of unisexuality in the Malpighiaceae. This large, predominantly Neotropical family shows a wide range in growth forms and fruit types but a relatively constant floral morphology ("floral conservatism" of W. R. Anderson, 1979). Anderson's (1981) study of herbarium collections led him to hypothesize that *Spachea elegans* (G. F. W. Meyer) Adr. Jussieu and *S. tricarpa* Adr. Jussieu, predominantly South American species, are gynodioecious or functionally dioecious. Collections with mature fruits had flat unopened anthers, and plants with pollen-bearing anthers never had enlarging fruits on old inflorescences (W. R. Anderson, 1981). Both Croat (1978) and Cuatrecasas and Croat (1980) failed to mention the possibility of gynodioecism or functional dioecism in the related *Spachea membranacea* Cuatr., which occurs in tropical moist forests of Panama and Colombia (Cuatrecasas and Croat, 1980). The purpose of this study was to document whether populations of *S. membranacea* on Barro Colorado Island (BCI) in central Panama exhibited a floral dimorphism analogous to that observed by W. R. Anderson (1981) and if so, to determine whether they were functionally gynodioecious or dioecious.

Spachea membranacea is a locally common understory tree (up to 10 m) on BCI that bears small (1-cm diam) pink flowers on pendent racemes during the early wet season (May–June). The flowers consist of five sepals, four of which bear paired oil-secreting glands (cf. elaiophores of Vogel, 1974; W. R. Anderson,

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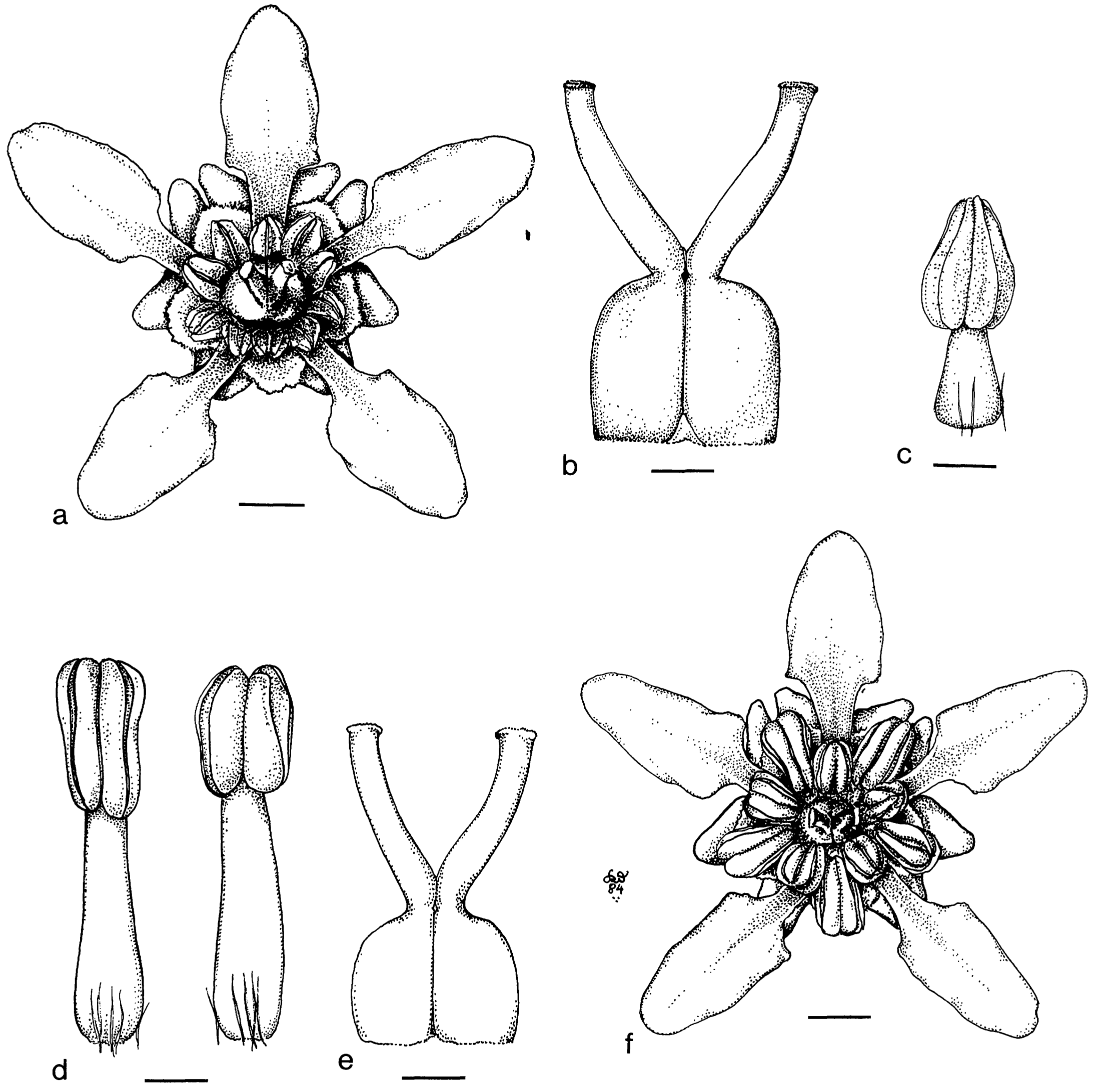

Fig. 1. *Spachea membranacea*. **a–c.** Female flower, gynoecium, nonfertile stamen; scale lines: a) 1 mm; b) 0.5 mm; c) 0.5 mm. **d–f.** Stamens, gynoecium, hermaphrodite flower; scale lines: d) 0.5 mm; e) 0.5 mm; f) 1 mm.

1979), five separate petals, 10 stamens, and an ovary that usually (but see Results) contains two ovules (Fig. 1).

METHODS AND STUDY SITE—Barro Colorado Island, the site for this study, is a 12 km^2 nature reserve situated in Lake Gatun, a part of the Panama Canal. *Spachea membranacea* is locally common in the young forest (ca. 70 yr) on BCI (Steiner, pers. obs.) but is only occasionally found in old forest (100+ yr). One small population, referred to as the Plateau population, has been recorded from a mapped 50 ha plot in the old forest (Hubbell and Foster, unpubl.). This population, and portions of populations at BCI trail locations called Pearson 8, Shannon 13, and Van Tyne 12, were studied during May and June 1983. Plant voucher specimens of the individuals studied are at DAV, while bees have been deposited at the Los Angeles County Museum.

Floral morphology—Two types of trees were found on BCI, those with morphologically hermaphroditic flowers with normal-looking styles, ovules, and anthers bearing pollen, and those with flowers that looked similar but lacked pollen-bearing anthers (females). Ovary and ovule size, style length, and stamen length were measured on five flowers from four hermaphrodite and four female trees in the Pearson population and from seven hermaphrodite and four females from other populations. Ratios of hermaphrodite to female trees were determined for the Plateau, Pearson, and Shannon populations, while diameters at breast height (DBH's) were recorded for the Plateau and Shannon populations.

Experimental pollinations—Five pollination treatments were performed on hermaphroditic flowers. Flowers were left either unmanipulated and unbagged (=open pollination) or unmanipulated but enclosed in a fine mesh nylon bag, or self-pollinated and bagged, or cross-pollinated after emasculation and bagged, or cross-pollinated without emasculation and bagged. Female flowers were either unmanipulated and bagged or cross-pollinated and bagged.

Floral visitors—Floral visitors were observed and collected primarily in conjunction with the pollination experiments. These observations spanned the entire flowering period and totaled about 20 hr but involved mainly hermaphrodites at the Plateau, Pearson, and Shannon sites.

RESULTS—*Floral characteristics*—*Spachea membranacea* flowers open between 0530 and 0630 local time. If left unpollinated, the entire hermaphrodite flower abscises after 1 day, but only the petals of the female flower abscise after the first day. The remainder of the female flower abscises after 2–3 days. An average hermaphrodite inflorescence has 47 flowers (N = 30, range: 26–75) with a variable number of flowers opening each day over a 5–10 day period. Individual trees flower for 3–4 wk, while a population will flower for 4–5 wk.

Hermaphrodite flowers have smaller ovaries and ovules, longer styles and stamens than female flowers in the Pearson population (Table 1). Except for style length (in the Plateau population females had longer styles than hermaphrodites), these differences were characteristic of other populations as well. The anthers and stigmas of hermaphrodite flowers are at the same position in the flower, but the stigmas extend beyond the shorter sterile stamens in female flowers (Fig. 1). Although the ovules of hermaphrodite and female flowers look essentially the same, ovules from hermaphrodite flowers (with one exception) were always smaller.

In the generic description, Cuatrecasas and Croat (1980) note that the ovary can be two- or three-loculate and ovulate, but variability of this sort is not mentioned under the description of *S. membranacea*. Both three- and one-loculate and ovulate flowers were found in low frequency on BCI. Five of 103 (4.9%) female flowers examined had three carpels and ovules (3 of 13 trees), while only 1 of 310 (0.3%) hermaphrodite flowers (1 of 20 trees) showed this character. Cases where the carpel and ovule number were reduced to one were found in 3 of 310 (1%) hermaphrodite flowers (3 of 20 trees), but none were found in female flowers (0/103, 13 trees).

TABLE 1. Spachea membranacea *flower size (mm) comparisons within the Pearson population on BCI. Five flowers from each of four hermaphrodites and four females were measured*

Hermaphrodites	Female	F-ratio	P
Ovary length			
1.38 ± 0.15	1.50 ± 0.14	6.6	<.01
Ovule length			
0.90 ± 0.09	1.16 ± 0.08	87.9	<.001
Style length			
1.68 ± 0.14	1.55 ± 0.23	4.72	<.05
Stamen length			
3.08 ± 0.21	1.83 ± 0.17	425.2	<.001

The proportion of hermaphrodite and female trees varied between populations. The Plateau population was strongly biased in favor of hermaphrodites (87.5%, N = 16), while the Pearson and Shannon populations had nearly equal proportions with slightly more hermaphrodites at Pearson (60%, N = 10) and more females at Shannon (55.3%, N = 47).

The distribution of hermaphrodites and females in DBH size classes was quite similar (Fig. 2), suggesting that sexual expression is genetically fixed within an individual and is not related to size or age. Many of the females examined had retained some fruits from the previous year, providing additional evidence for sexual constancy.

Experimental pollinations—Hermaphrodite flowers normally last a single day before they abscise, but after emasculation and cross-pollination some flowers remained for 3–4 days. Fruiting success was, therefore, not scored until at least 1 wk following pollination.

Results of the experimental pollinations show that only emasculated hermaphrodites and female flowers that were cross-pollinated produced fruit (Table 2). Cross-pollinated female flowers resulted in significantly more fruit than all other treatments, while emasculated, cross-pollinated hermaphrodite flowers produced significantly more fruit than all treatments except 4 and 7 (Table 2). This was probably due

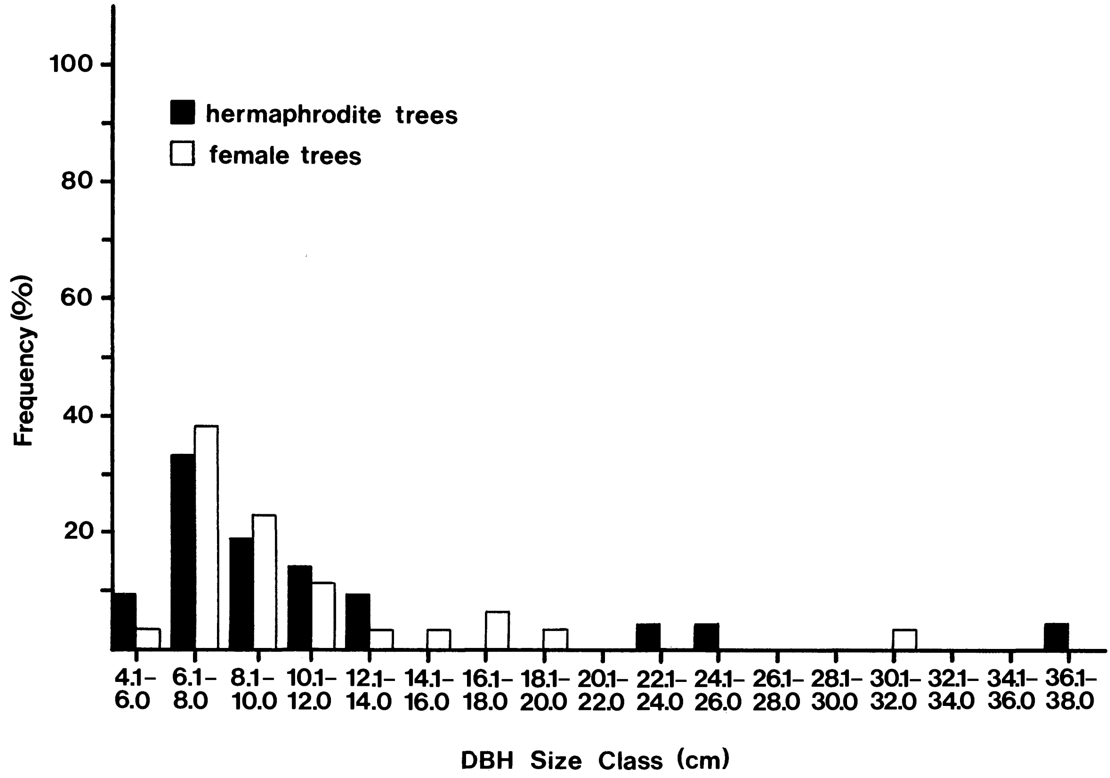

Fig. 2. Size class distribution of female and hermaphrodite trees of *Spachea membranacea* in the Shannon population on BCI ($N = 47$).

to the small sample sizes used in Treatments 4 and 7. Even though the sample size for non-emasculated, cross-pollinated hermaphrodites (Treatment 4) was small, consisting of flowers from a single tree, the same tree when emasculated prior to crossing had the highest fruit set (70%, $N = 30$) of the trees sampled. Variability in the success of emasculated cross-pollinations among hermaphrodite trees was quite high. Fruit set per tree ranged from 0–70% ($\bar{x} = 15.7\%$, SD = 26.8%, $N = 7$) with only three of the seven trees producing fruits.

Floral visitors—Visitation rates to *Spachea membranacea* were very low considering the large number of available flowers. The two most commonly observed visitors to *Spachea membranacea* flowers were *Paratetrapedia calcarata* Cresson (body length = 8.5 mm) and *Trigona pallens* Fabricius (body length = 6.0 mm), but *Paratetrapedia xanthaspis* Cockerell, *P. gigantea* Schr., *T. fuscipennis* Friese, *T. fulviventris* Guerin, and *Centris longimani* Fabr. also visited flowers. Both *P. calcarata* and *T. pallens* collect pollen and oil from hermaphrodite flowers; however, the oil collection behavior of these two bees differs. *P. calcarata* collects oil by scraping the calyx glands with its special foreleg basitarsal scraper setae (Neff and Simpson, 1981), while *T. pallens,* which lacks the modified setae, scrapes the glands with its mandibles (cf. Vogel, 1974). Both bees then transfer the oil to their hind scopae, where it is carried either alone or admixed with pollen to the nest.

Most pollinator observations took place in the Plateau population where there were only two poorly flowering female trees. Since no bees were observed to visit female flowers, it is not possible to compare foraging on the two floral morphs. The presence of oil in female flowers, however, suggests a reward sufficient to attract both *P. calcarata* and *T. pallens.*

The much larger bee, *Centris longimani* (body length = 21 mm) was observed systematically visiting hermaphrodite flowers once at the Plateau site and twice at the Shannon site. At the Plateau site, a single bee visited flowers on the tallest and most heavily flowering tree over a 4-hr period, but it did not move to surrounding trees, which were smaller and had fewer flowers. At the Shannon site, two *Centris longimani*

TABLE 2. *Crossing results for* Spachea membranacea *on Barro Colorado Island*

Treatment	Fruits/flowers	% Success	No. of trees
1. Unmanipulated hermaphrodite	0/522	0.0	9
2. Self-poll. hermaphrodites	0/132	0.0	4
3. Open-poll. hermaphrodites	0/3054	0.0	6
4. Cross-poll. herm., no emasculation	0/10	0.0	1
5. Cross-poll. herm. + emasculation	33/184	17.9	7
6. Cross-poll. females	24/41	57.7	1
7. Unmanipulated females	0/8	0.0	1

Significance levels from Chi-square two-way contingency tables:

Treatment 5 vs. Treatments 1, 2, 3, 6	$P < .001$
Treatment 5 vs. Treatment 4	$P > .10$
Treatment 5 vs. Treatment 7	$P > .10$
Treatment 6 vs. Treatments 1, 2, 3	$P < .001$
Treatment 6 vs. Treatment 4	$P < .025$
Treatment 6 vs. Treatment 7	$P < .05$

bees were observed collecting either pollen (short visits) or oil (longer visits) from two hermaphrodite trees.

DISCUSSION—The evolution of gynodioecism is presumed to occur when a male sterile mutant becomes favored in a population of hermaphrodites (Ross, 1982). For this to occur, the seed fitness of the mutants (females) must be twice that of the hermaphrodites to compensate for the lack of reproduction through pollen (Lewis, 1941). The ability of male sterile mutants to spread through a population of hermaphrodites may be explained by the selective advantage of increased outcrossing (Lloyd, 1982). Although selection for strict femaleness has been intense in the evolution of gynodioecism (Lloyd, 1982), this has not been the case with female sterility in the evolution of subdioecism and dioecism. Selection for female sterility appears to be much less intense in hermaphrodites of gynodioecious species, as many show substantial variation in their degree of female sterility (Arroyo and Raven, 1975; Lloyd, 1975; Webb, 1979; Atsatt and Rundel, 1982). The result is a spectrum of functional or physiological types ranging from full female fertility (functional gynodioecy) to total female sterility (functional dioecy), all within the morphological guise of gynodioecism. The problems of reconciling physiological and morphological breeding system indicators within a rigid system of classification is discussed by Bawa and Beach (1981).

Spachea membranacea is at the functionally dioecious end of the gynodioecious spectrum as morphological hermaphrodites fail to set seed, functioning solely as males. Only through emasculation can one induce fruit formation from some "hermaphrodite" flowers. No fruits were observed developing on any nonmanipulated hermaphrodites, but until further comparisons between emasculated and nonemasculated cross-pollinations can be made, the relative importance of emasculation and cross-pollination cannot be firmly established. The lack of fruit development on open pollinated hermaphrodites argues against cross-pollination as the factor governing induced fruit development, since fruits developed on open-pollinated females in the Pearson population where pollinators, even on hermaphrodites, were not commonly observed.

The mechanism by which emasculation influences seed development remains unknown, but the role of hormones in the control of sexual expression is well established even though the details vary from plant to plant. Removal of the stamens in hermaphrodite flowers of *Spachea membranacea* must upset the hormonal balance responsible for the maintenance of female sterility and thereby allow fruit development to take place.

Pollination—The low visitation rates by pollinators to *Spachea membranacea* may have been the result of competition for pollinators from other flowering species and/or reduced bee populations due to a long and unusually severe dry season. Bee populations on other flowering Malpighiaceae species also appeared low compared to visitation rates in 1980 (Steiner, unpubl.).

Most dioecious species of tropical forests are pollinated by small, generalist, social and solitary bees in the families Halictidae, Megachilidae, and/or Apidae (Bawa, 1980). The flowers of the plants visited by these bees are correspondingly small and lack morphological specializations that might suggest pollinator specificity. Although *Spachea* flowers are also small, they possess the specialized oil-secreting calyx glands typical of most Neotropical Malpighiaceae (Vogel, 1974).

There is a close association between Neotropical Malpighiaceae and oil-collecting anthophorid bees in the genera *Centris, Epicharis,* and *Paratetrapedia* (Vogel, 1974; Neff and Simpson, 1981; Simpson and Neff, 1981, 1983). *Centris* and *Epicharis* preferentially visit canopy trees and lianas (Frankie et al., 1983), the predominant habit among Panamanian Malpighiaceae (Cuatrecasas and Croat, 1980). These medium to large bees (>12 mm) are rarely observed foraging in the forest understory (Steiner, unpubl.), which may explain why they were not commonly observed on *Spachea. Paratetrapedia* species, which are much smaller bees (5–10 mm), visit a wide variety of plants with small flowers in forest understory as well as more open forest edge habitats (Steiner, pers. obs.). They also tend to spend longer on a particular plant than more mobile larger bees. This tendency to restricted foraging is typical of the pollinators of many dioecious tropical trees (Bawa, 1980) and may explain why the only malpigs with unisexual flowers are understory trees that are presumably pollinated primarily by these and *Trigona* bees. *Malpighia romeroana* Cuatr. (Malpighiaceae), an understory shrub on BCI with small pink flowers similar to those of *Spachea,* is also pollinated by *Paratetrapedia* and *Trigona* spp.; however, it shows no evidence of unisexuality (Steiner, unpubl.).

Evolution of dioecism—One would predict, over evolutionary time, a divergence in the morphologies of the sexual parts in female and hermaphrodite (functionally male) *Spachea* flowers based on sexual selection and resource allocation arguments (cf. Ross, 1982). With elimination of the male function in female flowers, ovules should become larger and/or more numerous, and stamens should become reduced. Functional male flowers should develop larger stamens with more pollen and smaller and/or fewer ovules. Such is in fact the case for *S. membranacea* where female flowers have larger ovules and smaller stamens while functional male flowers have smaller ovules and larger stamens.

The difference between the two flower types in *Spachea membranacea* is also evident from the proportions of one- and three-carpelate flowers observed. The frequency of female flowers that had three carpels (three ovules) was much larger than that for functional males, while only in functional males were cases of a single carpel (ovule) found. It is somewhat surprising that the functional males have retained viable ovules and the ability to set fruit occasionally (albeit with physiological modification); however, this supports the notion that selection for female sterility has been much less intense than selection for male sterility in the evolution of dioecism from gynodioecism (Lloyd, 1982).

Two additional *Spachea* species have been examined and shown to be morphologically gynodioecious and probably functionally dioecious (W. R. Anderson, 1981), and in both cases, ovules are present in the "hermaphrodite" flowers. Based on the drawings in Anderson (1981), the size difference between the ovaries in hermaphrodite and female flowers in *Spachea elegans* is even greater than it is in *S. membranacea,* yet it remains to be seen whether the physiological capability to set fruit has been retained in this and other species of *Spachea.* Although one might expect different *Spachea* species to occupy different positions on the morphological and/or functional spectrum between gynodioecy and dioecy, there is no evidence to suggest that differentiation along this axis has occurred. Apparently evolutionary divergence, which has resulted in the recognition of distinct morphological species within the genus, has occurred without a concomitant divergence in breeding system. Clearly, a more detailed look at the functional breeding systems of the remaining *Spachea* species is in order.

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