Floral dimorphism, pollination, and self-fertilization in gynodioecious *Geranium richardsonii* (Geraniaceae)[1]

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The selective maintenance of gynodioecy depends on the relative fitness of the male-sterile (female) and hermaphroditic morphs. Females may compensate for their loss of male fitness by reallocating resources from male function (pollen production and pollinator attraction) to female function (seeds and fruits), thus increasing seed production. Females may also benefit from their inability to self-fertilize if selfing and inbreeding depression reduce seed quality in hermaphrodites. We investigated how differences in floral resource allocation (flower size) between female and hermaphroditic plants affect two measures of female reproductive success, pollinator visitation and pollen receipt, in gynodioecious populations of *Geranium richardsonii* in Colorado. Using emasculation treatments in natural populations, we further examined whether selfing by autogamy and geitonogamy comprises a significant proportion of pollen receipt by hermaphrodites. Flowers of female plants are significantly smaller than those of hermaphrodites. The reduction in allocation to pollinator-attracting structures (petals) is correlated with a significant reduction in pollinator visitation to female flowers in artificial arrays. The reduction in attractiveness is further manifested in significantly less pollen being deposited on the stigmas of female flowers in natural populations. Autogamy is rare in these protandrous flowers, and geitonogamy accounts for most of the difference in pollen receipt between hermaphrodites and females. Female success at receiving pollen was negatively frequency dependent on the relative frequency of females in populations. Thus, two of the prerequisites for the maintenance of females in gynodioecious populations, differences in resource allocation between floral morphs and high selfing rates in hermaphrodites, occur in *G. richardsonii.*

Key words: floral dimorphism; frequency-dependent selection; geitonogamy; Geraniaceae; *Geranium richardsonii*; gynodioecy; pollen receipt; pollinator choice; self-fertilization.

Gynodioecy, a mode of sex expression in which separate hermaphroditic and female plants coexist in populations, was defined by and has been a topic of evolutionary interest since the work of Darwin (1877). Although the majority (~75%) of flowering plant species produce only hermaphroditic flowers, gynodioecy is a relatively common condition, having been described in almost 7% of the 121 492 species surveyed by Yampolsky and Yampolsky (1922). It is particularly prevalent in temperate floras, but rare in the tropics (Bawa, 1979; Richards, 1986). In natural populations, gynodioecy is typically controlled by a combination of cytoplasmic genes producing male-sterile (hereafter female) plants and nuclear genes restoring male function (Ross, 1978; Richards, 1986; Couvet et al., 1990). Gynodioecy is thought to be the most likely intermediate evolutionary step from hermaphroditism to dioecy (Darwin, 1877; Lloyd, 1975; Charlesworth and Charlesworth, 1978; Ross, 1978; Bawa, 1980).

Plants with hermaphroditic flowers can contribute genes to the next generation through both male and female function (pollen and seeds, respectively). Female plants of gynodioecious species contribute genes only through seeds. Because the male and female reproductive contribution to the next generation must balance (Fisher, 1930; Charnov, 1982), both sexual types can only be maintained in populations by natural selection if female plants compensate with an increase in female reproductive success. Reproductive compensation by females for loss of male fitness may occur by (1) increased seed production, and/or (2) increased seed or offspring quality of females relative to hermaphrodites. Females may increase seed production by reallocation of resources away from male function (pollen production and pollinator attraction) to female function (seeds and fruits). Females may also benefit from their inability to self-fertilize if selfing reduces seed set and offspring quality in hermaphrodites relative to females (Darwin, 1877; Lewis, 1941; Lloyd,

1975, 1976; Ross, 1978; Charlesworth and Charlesworth, 1978; Charlesworth and Ganders, 1979; Charlesworth, 1981; Charnov, 1982).

The female flowers of almost all gynodioecious species are smaller than those of hermaphrodites (Darwin, 1877; Baker, 1948; Delph, 1996). This suggests that resource allocation from male to female function may be an important factor favoring females. However, the reduction in flower size in females is expected to have negative impacts on pollen receipt, which must be weighed against the potential advantages of redirecting resources to fruit and seed production (Charlesworth and Charlesworth, 1987). Large flower and inflorescence size are positively associated with pollinator attraction in many species (Bell, 1985; Geber, 1985; Ashman and Stanton, 1991; Eckhart, 1991; Roberston, 1992; de Jong, Waser, and Klinkhammer, 1993; Schemske and Agren, 1995; Snow et al., 1996), and greater attractiveness to pollinators may promote male reproductive success (RS) more strongly than female RS (Bell, 1985; Stanton, Snow, and Handel, 1986; Mitchell, 1993; Queller, 1997; but see Wilson et al., 1994; Broyles and Wyatt, 1990, 1997). In the seven studies of gynodioecious species reviewed by Delph (1996), pollinators preferentially visited the larger hermaphroditic flowers, although fruit set in females did not appear to be pollen limited.

In gynodioecious species, hermaphrodites may be fertilized by self-pollen, either through autogamy (within-flower selfing) or geitonogamy (selfing between flowers on the same plant). If self-pollination results in a reduction in offspring number or quality because of inbreeding depression or pollen interference (de Jong et al., 1993; Snow et al., 1996), then females, which are obligately outcrossed, will gain a fitness advantage over hermaphrodites (Lloyd, 1975; Charlesworth and Charlesworth, 1978). Autogamy is reduced in many hermaphroditic flowers by the spatial (herkogamy) or temporal (dichogamy) separation of male and female reproductive structures (Lloyd and Webb, 1986; Webb and Lloyd, 1986; Richards, 1986). Geitonogamy is not so easily avoided in plants producing multiflowered displays. Although larger inflorescences are more attractive to pollinators, large floral displays have a greater probability of geitonogamy (reviewed by de Jong et al., 1993; Harder and Barrett, 1996; Snow et al., 1996).

Factors affecting the relative fitness of females and hermaphrodites may vary according to the environmental context (e.g., resource and pollinator availability) (Ashman and Stanton, 1991; Eckhart, 1991, 1992a; Ashman and Baker, 1992), as well as the demographic context in which they grow. In particular, pollen availability to females may depend on the density and frequency of pollen-producing hermaphrodites in the population. The frequency of female plants in gynodioecious populations may vary widely over relatively short distances (Couvet et al., 1990). As females become more common in a population, they may suffer increasing pollen limitation. The stable frequency of females in populations of gynodioecious plants might therefore be expected to be under density- or frequency-dependent control (Lloyd, 1975; McCauley and Taylor, 1997, McCauley and Brock, 1998).

In this paper we report on some of the factors influ-encing pollination and outcrossing success that may maintain gynodioecy in populations of *Geranium richardsonii* in Colorado. We examine the following questions. (1) Does floral dimorphism occur between female and hermaphroditic flowers? (2) Does floral dimorphism vary among populations and habitats? (3) How do differences between floral morphs affect pollinator attraction and pollen receipt? (4) What proportion of the pollen received by hermaphrodites is the result of autogamous or geitonogamous pollination? (5) Is the pollination success of females dependent on their density or frequency in a population?

MATERIALS AND METHODS

Study organism—*Geranium richardsonii* Fischer and Trautvetter (Geraniaceae) is a long-lived perennial native to moderately disturbed habitats in the subalpine zone. Plants are typically multistemmed and produce numerous five-merous flowers. The number of flowers open per day per plant varies widely (range 0–70; $\bar{X}$ = 20.4, SD = 11.6, N = 40) and depends on plant size and locality. The flowering season extends of over several weeks (typically late June through early August in the study area). Flowers of *G. richardsonii* are radially symmetrical with white petals and pink to purple veins (Weber, 1987). Flowers are visited by a wide array of potential pollinators including bumble bees, solitary bees, and several families of flies (Kearns, 1990).

Geranium richardsonii is gynodioecious; individual plants produce either hermaphroditic or female flowers. The perfect flowers of the typical hermaphroditic plant are protandrous, producing ten fertile stamens in two whorls. Filaments are as long, or longer than, the style. Anthers of hermaphroditic flowers are large and purple, producing hundreds of large (75–100 μm diameter) pollen grains. Flowers remain in male phase (pollen dehiscence) for 1–2 d before the five stigma lobes split and expose the papillate stigmatic surface. By this time the stamens have reflected back toward the petals, and many of the anthers may have been lost. Flowers remain in female phase for ~1 d before the stigma lobes close and the petals shrivel and drop. The flowers of female plants are characterized by small, yellow anthers on short filaments. These anthers produce either no pollen or small, inviable grains. Stigma lobes are open and exposed soon after the flowers open and remain open for 2–3 d, until after the petals wilt and drop. The female phase is thus considerably longer in female than hermaphrodite flowers.

Study area and population characteristics—We conducted our research in the vicinity of the Rocky Mountain Biological Laboratory (RMBL) in the East River Valley near Gothic, Colorado (2900 m). Snowmelt typically occurs from late April to early June at this subalpine site. Aspen forest supports a diverse understory in which *G. richardsonii* is common, averaging ~3% of the total cover (Langenheim, 1962). Near the RMBL, *G. richardsonii* most commonly occurs at the margins of aspen and willow stands in moderately disturbed habitat and open meadows at higher elevations (above 3200 m). Spatially discrete populations of *G. richardsonii* occur throughout the area surrounding the RMBL. A survey of 13 populations within 2 km of RMBL revealed wide variation in the frequency of female plants. Female frequency varied from 0 to 62% among populations with a mean frequency of 22% (C. F. Williams, unpublished data). Between 1994 and 1998, observations and experiments were conducted in seven *G. richardsonii* populations near the RMBL spanning a range of habitats, plant densities, and sex ratios (Tables 1, 2).

Floral dimorphism—We measured hermaphrodite and female flower size from one or more populations in each of four years (1994–1997). In 1994–1996, we collected 1–11 flowers from each of 24–56 plants from three different populations. In 1996 we further grouped flowers and plants by whether they grew in sun (open meadow at forest edge)

TABLE 1. Flower density and sex ratio in populations used in the 1997 pollen deposition experiments at RMBL. All flowers on all plants in the spatially discrete populations were counted in each census period. Areas of populations are Pumphouse (96 m²), Enders (40 m²), Virginia (40 m²), and Aspen (120 m²).

Population	Census period	Sampling date(s)	Number of flowers		% Females	Density (no./m²)	
			Hermaph.	Females		Hermaph.	Females
Pumphouse	1	6/25–6/30	90	52	36.6	0.94	0.54
Pumphouse	2	6/30–7/5	422	277	39.7	4.40	2.89
Pumphouse	3	7/14–7/18	379	281	42.6	3.95	2.93
Pumphouse	4	8/1	149	85	36.1	1.55	0.89
Enders	2	6/30–7/5	42	89	67.9	1.05	2.23
Enders	3	7/14–7/19	474	184	28.0	11.85	4.60
Enders	4	8/8	295	136	31.6	7.38	3.40
Virginia	3	7/18–7/23	87	422	82.9	2.18	10.55
Virginia	4	8/9	22	48	68.6	0.55	1.20
Aspen	2	6/30–7/5	248	31	11.1	2.07	0.26
Aspen	3	7/14–7/19	275	44	13.8	2.29	0.37
Aspen	4	8/1	41	6	12.8	0.34	0.05

or shade (beneath canopy of aspen forest) habitats in the Research Meadow population. In 1997 we collected flowers haphazardly, not grouped by plant, from four different populations as part of the pollinator choice experiment described below. We measured the length of two petals from each flower to the nearest 0.01 mm with digital calipers. In each population sampled in 1994–1996 we analyzed differences in mean petal length per flower between hermaphrodite and female plants using a nested ANOVA (SuperANOVA v. 1.11; Abacus Concepts, 1991) with plants nested within floral morph. In 1996 we further tested for the effects of habitat (sun vs. shade) on mean petal size per plant using a two-way ANOVA, with habitat and morph as fixed effects. In 1997 we analyzed differences in mean petal size per flower between the two morphs with a two-way ANOVA, with floral morph as a fixed effect and population a random effect.

Pollinator choice—We examined the effect of floral sexual morph on pollinator visitation in artificial arrays. We conducted 13 30-min

TABLE 2. Mean (±1 SD) petal lengths of hermaphrodite and female flowers sampled at RMBL. Asterisks indicate statistical differences between floral morphs and among plants nested within morphs (Nested ANOVA; **$P < 0.001$, *$P < 0.01$) for the 1994–1996 data. In 1997 there was a significant effect of floral morph ($F_{1,3} = 473.3$, $P = 0.0002$), and population ($F_{3,303} = 7.74$, $P < 0.0001$) on mean petal length per flower. The interaction of morph and population was not significant ($F_{3,303} = 2.16$, $P = 0.314$).

Date	Population	Sex	N plants	N flowers	Petal length (mm)
1994	Pumphouse	Hermaph**	28**	60	13.33 (±1.72)
		Female	28	47	10.35 (±1.62)
1995	Pumphouse	Hermaph**	12*	26	15.51 (±1.06)
		Female	12	27	12.32 (±1.09)
1995	Levi	Hermaph**	17**	35	16.86 (±1.41)
		Female	15	36	12.62 (±1.69)
1996	Research Meadow	Hermaph**	16**	50	15.79 (±1.75)
		Female	21	47	11.57 (±1.21)
1997	Aspen	Hermaph	—[a]	11	17.70 (±1.33)
		Female	—	12	13.10 (±1.61)
1997	Enders	Hermaph	—	49	17.11 (±1.32)
		Female	—	49	12.89 (±0.87)
1997	PH	Hermaph	—	49	17.18 (±1.51)
		Female	—	44	12.50 (±1.46)
1997	Virginia	Hermaph	—	47	17.71 (±1.45)
		Female	—	48	13.72 (±1.34)

[a] Flowers were not grouped by plant for analysis in 1997.

trials in four different populations between 14 and 31 July 1997. Arrays consisted of 25 *G. richardsonii* flowers picked haphazardly from throughout each study population and arranged haphazardly in a 5 × 5 grid. We placed individual cut flowers in numbered, water-filled, 5-mL shell vials glued to a board 7.5 cm apart. Each array contained 13 flowers of one morph and 12 of the other. We placed the array in the population with the flowers at approximately the same height as the surrounding flowers on intact plants. During each 30-min observation period, we recorded the following behaviors for each flower visited: flower number, pollinator type (fly, small bee, bumble bee, butterfly, beetle), duration of visit, and subsequent destination (flower within the array or leave array). We compared the number of visits to hermaphrodite and female flowers to the expected 13:12 ratio for goodness of fit for each trial separately. We tested the overall fit to the expected ratio in all trials combined and heterogeneity among trials with a *G* test of heterogeneity (Sokal and Rohlf, 1981). We used a two-factor, fixed-effect ANOVA to examine whether the mean duration of pollinator visits differed between floral morphs and trials.

Pollen deposition—*Autogamy experiment*—To estimate the proportion of autogamous self-pollination, we compared pollen deposition between emasculated and unemasculated flowers on the same hermaphroditic plants. We also examined whether pollen receipt differed between hermaphrodite and female flowers as an estimate of the "cost" of male sterility. We repeated each autogamy experiment 2–4 times in each of four populations over the course of the flowering season. We chose populations that spanned a range of plant density and female plant frequency. Both population size (number of flowering plants) and morph frequency (% female flowers) also varied throughout the season within each population (Table 1). We compared pollen deposition among three treatments: unemasculated (U) and emasculated (E) flowers on hermaphroditic plants, and unmanipulated flowers on female plants (F). The remaining "non-experimental" flowers on hermaproditic plants were left unemasculated. We performed emasculations prior to or very early in anthesis by removing all anthers with fine forceps. We performed both U and E treatments on a small number of the total flowers on each hermaphroditic plant. Each hermaphroditic plant therefore had both treatments (U and E), while female plants had only a single treatment (F). In each population, during each sampling period, we marked 15–129 flowers per treatment ($\bar{X} = 59.4$ U, 35.5 E, and 49.0 F) using small paper jewelry tags tied around the peduncle. We marked and emasculated flowers over a 2–5 d period and checked them daily for collection at the end of flowering, soon after they lost their petals. In the laboratory, we removed the stigmas with fine forceps and squashed them on microscope slides in basic fuchsin gel (Kearns and Inouye, 1993). We counted stained pollen grains adhering to stigmas under a compound microscope at 100×.

Because the U and E treatments are on the same plants, they are not independent. Therefore, we performed two separate analyses to test differences: (1) between unemasculated and emasculated (U vs. E) on the same plants, and (2) between hermaphrodite and female flowers ([U + E] vs. F) on different plants. To test for differences in pollen deposition between treatments in hermaphroditic plants (U vs. E), we used a two-way, mixed-model ANOVA, where treatment is a fixed effect and plant a random effect. To test the differences in pollen deposition between floral morphs, we used a nested ANOVA, with floral morph (hermaphrodite vs. female) as a fixed effect and plant nested within morph a random effect. Because phenological differences might lead to variation in pollen availability during the different sampling periods, we performed separate analyses for each population in each sampling period.

Geitonogamy experiment—The autogamy experiment described above did not control for the possibility of geitonogamous self-pollination inflating pollen deposition in hermaphrodites. Geitonogamy results if self-pollen is received by hermaphroditic flowers from other unemasculated flowers on the same plants. To estimate the contribution of geitonogamy to self-pollination, we compared pollen deposition on stigmas of emasculated "target" flowers on plants with all their remaining "non-target" flowers either unemasculated or emasculated. We repeated this experiment twice, from 1 to 8 August 1997 and from 13 to 21 July 1998, in the Oh-Be-Joyful population. Flowering plant density and morph frequency were not quantitatively assessed in this population, although it was composed predominantly (>95%) of hermaphroditic plants. We randomly assigned 20 hermaphroditic plants to each of the two plant treatments, unemasculated or emasculated. We also marked 8–10 female plants. All open flowers were emasculated each morning on the 20 emasculation-treatment plants throughout the course of the experiments. If emasculation-treatment plants grew in close proximity to other hermaphrodites, we emasculated all flowers on surrounding plants within a 0.5-m radius of the focal plants to avoid pollen contamination that would confound estimates of geitonogamy. On each of 20 unemasculated plants we marked and emasculated 2–9 target flowers to examine pollen deposition (U treatment = emasculated flowers on unemasculated plants). On these same unemasculated plants, we also marked and collected 4–12 unemasculated flowers as controls for autogamous self-pollination (C treatment = unemasculated flowers on unemasculated plants). On each of 20 emasculated plants, we marked and emasculated 2–9 target flowers (E treatment = emasculated flowers on emasculated plants). Five to 15 flowers from female plants were also collected to compare pollen deposition between females and hermaphrodites (F = unmanipulated female flowers on female plants). Differences between the C and U treatments are an estimate of autogamy. Differences between the U and E treatments are an estimate of the proportion of pollen received from geitonogamous pollination. Finally, differences between female (F) and hermaphrodite treatments (C, U, and E) are an indication of effects of male sterility on pollen deposition.

Because C and U treatments were on the same unemasculated plants they were not independent. Therefore, we first analyzed the differences among the independent U, E, and F treatments using a nested ANOVA, where treatment was a fixed effect and plant nested within treatment a random effect. Pairwise differences were tested with contrasts of group means (SuperANOVA, v. 1.11; Abacus Concepts, 1991). We tested differences between the C and U treatments with a two-way ANOVA, where treatment was a fixed effect and plant a random effect. The results from 1997 and 1998 were tested separately.

Density- and frequency-dependent pollination success of floral morphs—We compared the relative success of hermaphrodite and female flowers at receiving pollen among populations differing in hermaphrodite flower density and floral morph frequency. During each sampling period in 1997 we censused the number of flowers of each morph within each population (Table 1). Relative pollination success per census was calculated as the ratio of average pollen deposition for

TABLE 3. Plant means (±1 SD) of petal length of hermaphrodite and female plants sampled in 1996 from sun and shade habitats in the Research Meadow population. There was a significant effect of sex ($F_{1,59} = 178.4$, $P < 0.0001$) and a marginally significant effect of habitat ($F_{1,59} = 3.83$, $P = 0.055$) on petal length. There was no significant interaction between the two factors ($F_{1,59} = 2.56$, $P = 0.115$).

Habitat	Sex	N plants	Petal length (mm)
Sun	Hermaph	13	15.51 (±1.67)
	Female	16	11.79 (±1.13)
Shade	Hermaph	17	16.63 (±0.81)
	Female	17	11.90 (±1.34)

females to unemasculated hermaphrodites. Density dependence was analyzed as the regression of mean pollen receipt (by hermaphrodites or females) on the density of hermaphrodites (number of flowers per square meter) in the population (Statview v. 4.1; Abacus Concepts, 1994). A significant positive correlation indicates that pollen availability is associated with pollen deposition in different populations over the course of the flowering season. Frequency dependence was analyzed as the regression of relative pollination success on the percentage of female flowers censused in the population. A significant negative correlation indicates that female pollination success is limited by the availability of pollen from hermaphroditic pollen donors in the population.

RESULTS

Floral dimorphism—Flower size (petal length) was significantly greater in hermaphrodites than females in all populations studied (Table 2). Hermaphrodite petals were on average 1.32 times longer than in females. In 1994–1996, when flowers were nested within plants for analysis, there were significant differences among plants within floral morph in all populations. There was also significant heterogeneity in flower size among populations in the 1997 data (Table 2). One potential cause of flower size variation among plants within a morph was the environment in which different plants grow. In 1997, plants from shadier environments (Virginia and Aspen populations) tended to have slightly larger petals (Table 2). We tested for the effect of light environment by comparing flower size among plants growing in sun and shade habitats in a spatially continuous population at an aspen forest margin in 1996. Again, there was a highly significant effect of floral morph on average plant petal length (Table 3). The effect of habitat (sun vs. shade) was only marginally significant, with hermaphrodite petal length slightly larger in the shade habitat. The interaction between floral morph and habitat was not significant (Table 3).

Pollinator choice—We recorded 278 total insect visits in 130 bouts to artificial arrays during 13 trials (Table 4). Hermaphrodites were visited more frequently than females in 12 of the 13 trials. Overall, both the total and pooled tests of deviations from the random expectation of 1:1 for the 13 trials were highly significant, with a large excess of visits to hermaphrodites. Heterogeneity among trials was not significant (Table 4).

A variety of insects including worker bumble bees, solitary bees, flies, small beetles, and butterflies visited *G. richardsonii* flowers. All types of insects preferentially visited hermaphrodite flowers in the arrays. Bees and flies

TABLE 4. Visitation frequency of pollinators to hermaphrodite and female flowers in artificial arrays. Results of *G* tests for individual trials are shown at right. Results of heterogeneity *G* tests are shown below. Note that the sum of individual *G*s and G_{total} are not equal because the expected ratios differed slightly among individual tests.

Population	Observed visits		Expected ratio (H:F)	G
	Hermaphrodite	Female		
Pumphouse	8	5	13:12	0.48
Enders	8	6	13:12	0.15
Enders	3	2	12:13	0.29
Pumphouse	1	0	13:12	—
Virginia	8	1	12:13	6.77*
Aspen	7	2	13:12	2.56
Virginia	3	1	12:13	1.21
Enders	32	21	12:13	3.26*
Pumphouse	4	6	13:12	0.58
Virginia	12	5	13:12	2.43
Pumphouse	19	13	12:13	1.66
Virginia	36	10	12:13	17.75**
Enders	44	21	12:13	10.26**
Total	185	93	1:1	47.42

G tests	df	G	P
Pooled	1	31.028	<0.001
Heterogeneity	12	12.452	0.1–0.5
Total	13	43.480	<0.001

* Test significant at $P < 0.01$; ** test significant at $P < 0.001$.

visited more than one flower in the array in the majority of foraging bouts observed. The mean duration of individual flower visits also differed among pollinator types (Table 5). However, there was not a significant difference in the duration of visits per trial to hermaphrodite and female flowers (duration for hermaphrodites, $\bar{X} \pm 1$ SE $= 32.8 \pm 12.2$ s; duration for females 6.3 ± 1.1 s; $F_{1,252} = 0.750$, $P = 0.387$). The effect of trial and the interaction between trial and floral morph were not significant (trial: $F_{12,252} = 0.381$, $P = 0.970$; sex × trial: $F_{11,252} = 0.025$, $P = 0.944$). The large differences in duration of visits between hermaphrodites and females were due primarily to 30 visits by small beetles, which spent an average of 246 s exclusively at hermaphrodite flowers (Table 5). When these visits by beetles are omitted from the analysis the difference in visit duration between morphs is greatly reduced and remains nonsignificant (hermaphrodite $= 6.7 \pm 0.8$ s; female $= 6.3 \pm 1.1$ s; $F_{1,222} = 0.139$, $P = 0.709$). The effects of trial, but not the interaction between sex and trial are significant in this analysis (trial: $F_{12,222} = 6.172$, $P < 0.0001$; sex × trial: $F_{12,222} = 0.714$, $P = 0.725$).

Pollen deposition—*Autogamy experiment*—On average, hermaphrodites (U + E) received more pollen than females (F). However, pollen receipt differed significantly ($P < 0.05$) between floral morphs in only five of the 12 comparisons (Fig. 1). The number of pollen grains deposited on stigmas of emasculated (E) and unemasculated (U) hermaphrodite flowers did not differ significantly in any of the eight possible comparisons (Fig. 1). The amount of pollen deposited also varied throughout the flowering season, being generally highest in the census corresponding to the highest flowering plant density (Fig. 1, Table 1).

TABLE 5. Flower visitation patterns of potential pollinators to *Geranium richardsonii* arrays. Percentage multiple visits are the number of total visits that were part of multiflower runs within the array by the same individual visitor.

Visitor type	Total no. of visits	Hermaphrodite flowers visited (%)	Mean duration of visits (s)	Multiple visits (%)
Bumble bees	97	62	2.43	94
Solitary bees	132	65	26.98	62
Flies	34	67	7.8	52
Beetles	30	100	245.9	20
Butterflies	3	67	2.67	0

Geitonogamy experiment—In 1997 and 1998 there were no significant differences between the control (C) and unemasculated (U) flower treatments on unemasculated plants (Fig. 2; 1997: $F_{1,19} = 2.62$, $P = 0.122$; 1998: $F_{1,19} = 1.24$, $P = 0.286$). In both years there were significant differences among plants (1997: $F_{19,171} = 2.25$, $P = 0.003$; 1998: $F_{19,316} = 4.41$, $P < 0.0001$), but no significant plant × treatment interaction (1997: $F_{19,171} = 1.53$, $P = 0.080$; 1998: $F_{19,316} = 1.15$, $P = 0.300$). In 1997, there were no significant differences among the three independent treatments (U, E, and F; Fig. 2) ($F_{2,45} = 1.35$, $P = 0.268$), although there was significant variation among plants nested within treatment ($F_{45,279} = 4.65$, $P < 0.0001$). In 1998, there were significant differences both among treatments ($F_{2,47} = 6.82$, $P = 0.0025$) and among plants within treatment ($F_{47,421} = 2.21$, $P < 0.0001$). Independent contrasts of group means
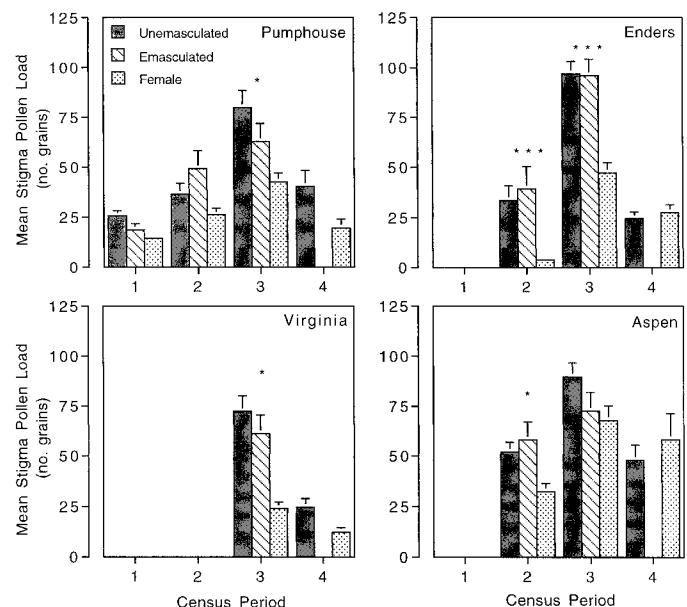

Fig. 1. Mean (± 1 SE) number of pollen grains deposited on stigmas of three flower treatments (U = unemasculated hermphrodite, E = emasculated hermaphrodite, and F = female) in each of four census periods in four populations of *Geranium richardsonii* in 1997. The remaining nonexperimental flowers on hermaphrodite plants were unemasculated. Asterisks over bars for each census indicate statistical differences between hermaphrodite (U + E) and female (F) treatments (*$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; nested ANOVA). Differences in pollen receipt between the U and E treatments on the same hermaphrodite plants were all nonsignificant (two-way ANOVA).
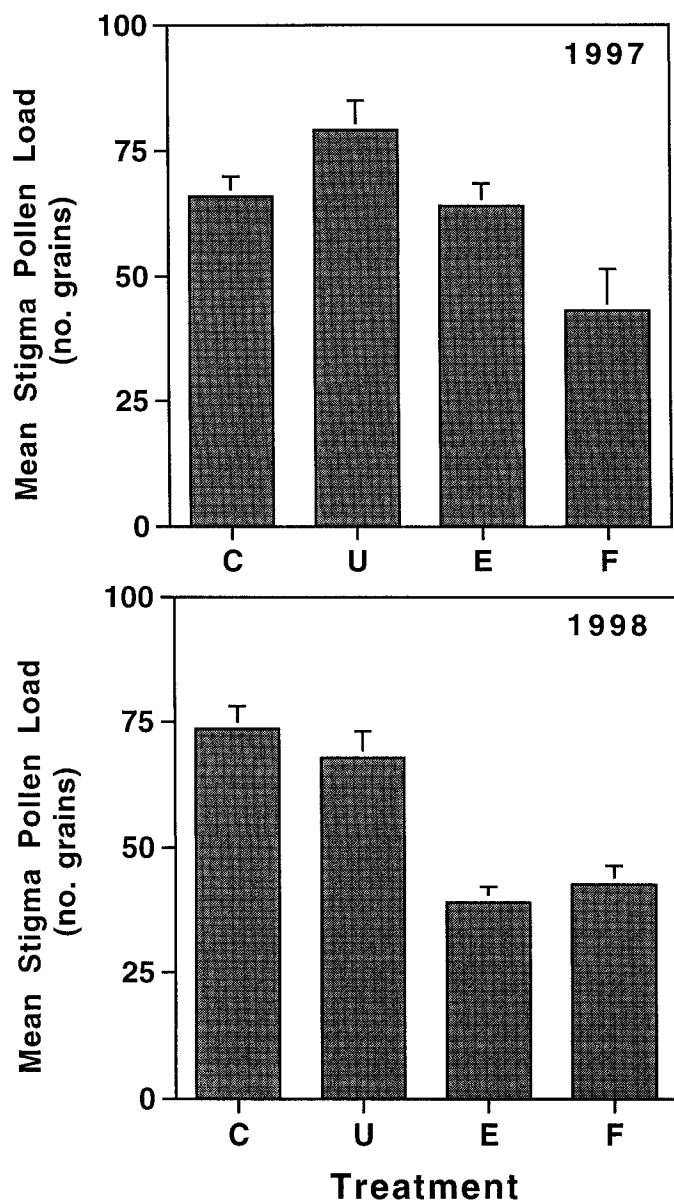
Fig. 2.   Mean ($\pm$1 SE) number of pollen grains deposited on stigmas of four treatments (C = unemasculated control flowers on enemasculated plants, U = emasculated flowers on unemasculated plants, E = emasculated flowers on emasculated plants, and F = unmanipulated female flowers) in the 1997 and 1998 geitonogamy experiments. In 1997, there were no significant differences among the four treatments. In 1998, there was no significant difference in pollen receipt between unemasculated (C) and emasculated (U) plants. There were significant differences among the U, E, and F treatments, with U receiving significantly more pollen than E and F. See text for statistical details.

indicated that U differed significantly from E and F, but E and F did not differ significantly from one another (U vs. E: $F_1 = 12.52$, $P = 0.0009$; U vs. F: $F_1 = 6.16$, $P = 0.017$; E vs. F: $F_1 = 0.311$, $P = 0.58$; Fig. 2).

Density- and frequency-dependent pollination success of flower morphs—There was a tendency for pollen receipt to increase in all flower types (Fig. 1) as the density of pollen producing hermaphroditic flowers increased
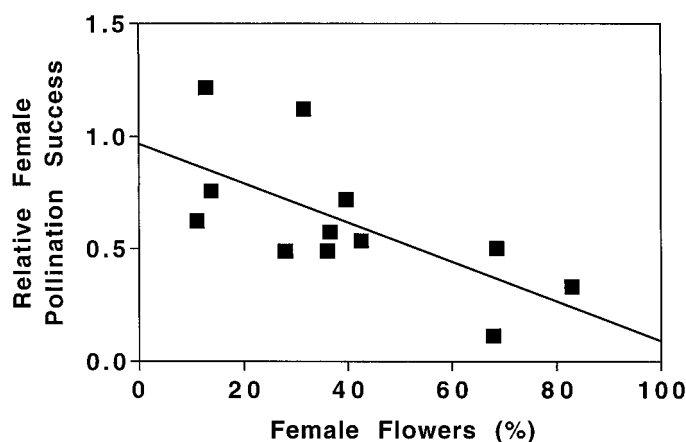

Fig. 3.   Relative success of female compared to unemasculated hermaphrodite flowers at receiving pollen in populations of different female frequency in 1997 censuses. Relative female success was calculated as the ratio of mean pollen receipt by female flowers to mean pollen receipt by unemasculated hermaphrodite flowers in each census.

(Table 1) in the four populations sampled in the autogamy experiment. However, the correlations were not significant between hermaphrodite flower density and the mean number of pollen grains deposited on stigmas of unmanipulated hermaphrodite flowers (Pearson $r = 0.436$, $N = 12$, $P > 0.05$), or female flowers ($r = 0.261$, $N = 12$, $P > 0.05$). There was a significant, positive correlation between hermaphrodite flower density and stigma pollen loads on emasculated flowers ($r = 0.766$, $N = 8$, $P < 0.05$). The relative success of female flowers at receiving pollen was a decreasing function of female frequency in populations. There was a significant negative correlation between relative female pollination success and the percent female flowers in the population ($r = 0.663$, $N = 12$, $P < 0.05$; Fig. 3). Female flowers in the most extremely female-biased populations had less than half the number of pollen grains deposited on their stigmas as did unmanipulated hermaphrodite flowers, indicating negative frequency dependence.

DISCUSSION

Floral dimorphism—*Geranium richardsonii,* like the majority of gynodioecious plant species studied to date, has hermaphroditic flowers significantly larger than its female flowers. Gynodioecy has been described in at least 11 other species of *Geranium* and *Erodium* (Geraniaceae), and all show this pattern of floral dimorphism (reviewed by Delph, 1996). The hermaphroditic flowers of *G. richardsonii* are on average 1.3 times larger than female flowers, similar to the value of 1.4 times found in its common Eastern North American congener *G. maculatum* (Agren and Willson, 1991). The phenotypic variation in flower size among plants within each sexual morph suggest that genetic variability for flower size exists in *G. richardsonii* populations. However, floral size variation also appears to result from environmental differences experienced by individual plants, seen in the sun vs. shade comparison (Table 3). Plants in shadier sites (e.g., Aspen and Virginia populations in 1997; Table 2) also tended to have somewhat larger petals. Petal size

responds much as do leaves, becoming smaller in sunny environments, particularly in hermaphrodites. Petals wilt more quickly and are retained for shorter intervals, in dry, sunny habitats (personal observation). Transpiration may place an upper limit on petal size and thus differences in resource allocation between hermaphrodite and female flowers.

Pollinator choice—The larger hermaphroditic flowers of *G. richardsonii* are visited more frequently by pollinators than female flowers. Previous studies of gynodioecious species have also shown higher visitation at hermaphroditic flowers (reviewed by Delph, 1996). Such pollinator preference for larger flowers may reflect a greater perceived reward (Eckhart, 1991; Ashman and Stanton, 1991; Schemske and Agren, 1995). Hermaphrodites in a number of gynodioecious species produce more nectar than females. In addition, pollen may also serve as a reward (reviewed by Delph, 1996). Many visitors to *G. richardsonii* collect both nectar and pollen (Kearns, 1990). Pollinator attraction is hypothesized to benefit male RS more than female RS since fewer visits are probably needed to maximize seed set than to remove and disperse pollen. Male RS may therefore be limited by mating opportunities, while female RS is resource limited (Bateman, 1948; Bell, 1985; Stanton, Snow, and Handel, 1986; Queller, 1997; but see Broyles and Wyatt, 1990, 1997; Wilson et al., 1994). The observed greater size and attractiveness of hermaphroditic flowers in most gynodioecious species support the view that floral size is an adaptation to enhance male reproductive function. Reductions in resources allocated to pollen production and pollinator attraction may allow for those resources to be reallocated toward greater seed production in female plants (Darwin, 1877; Lewis, 1941; Lloyd, 1975, 1976; Charlesworth and Charlesworth, 1978, 1987).

Greater lifetime female fecundity relative to hermaphrodites may result from lower plant mortality, more flowers per plant, and/or more seeds per fruit. Females in many gynodioecious species have been found to produce more seeds than hermaphrodites, although seldom the twofold advantage required for maintenance of male sterility required by some theory (e.g., Agren and Willson, 1991; Petterson, 1992; Eckhart, 1992b; Maki, 1993; and earlier studies reviewed by Couvet et al., 1990). Seed set per flower in *G. richardsonii* was on average only 1.2 times greater for females in five populations studied (C. Williams, unpublished data). This slight fecundity advantage is probably not sufficient to explain the maintenance of females in *G. richardsonii* and further fitness advantages must be sought.

Pollen deposition—Reallocation of floral resources from male function (pollen and petals) to female function (seed and fruit) may allow females to reproductively compensate for loss of male RS if smaller flower size does not reduce pollen receipt to the point of pollen limitation (Charlesworth and Charlesworth, 1987). Female flowers of *G. richardsonii* do receive significantly less pollen on their stigmas than hermaphrodites. However, females have seed set greater than or equal to hermaphrodites (see above), indicating that pollen loads are not so small as to be limiting. Other studies reviewed by

Delph (1996) also found that although visitation to female flowers was less than to hermaphrodite flowers, female RS was not pollen limited.

Although females receive a smaller quantity of pollen than hermaphrodites, this pollen may be of higher quality since it is necessarily outcrossed. On average, hermaphrodites received 2.2 times more pollen than females (calculated as the ratio of average number of pollen grains received by unemasculated hermaphrodites on unemasculated plants to those received by females in each of the 14 experiments; Figs. 1, 2). What portion of this additional pollen can be attributed to greater pollinator attraction to the larger hermaphroditic flowers vs. that received as the result of autogamous and geitonogamous self-pollination?

The results of both experiments comparing pollen receipt on emasculated and unemasculated target flowers on unemasculated plants (E vs. U in autogamy experiment, C vs. U in geitonogamy experiment) suggest that autogamy is uncommon. Unemasculated flowers received $< 1\%$ more pollen on average than emasculated flowers when averaged over all experiments. In no case were the differences between these treatments statistically significant. This result is perhaps not surprising since hermaphroditic *G. richardsonii* flowers are strongly protandrous. Such protandry suggests that avoidance of selfing has been a strong selection pressure in the evolution of *G. richarsonii*'s floral biology and mating system. Selfing can depress fitness in both self-compatible and self-incompatible species due to inbreeding depression and interference between self- and outcross pollen, respectively (reviewed by de Jong et al., 1993; Snow et al., 1996). Kearns (1990) inferred that *G. richardsonii* was self-incompatible or required a minimum threshold quantity of pollen for successful self-fertilization based on a limited number of hand crosses. Recent hand pollination experiments (K. O'Malley, C. Williams, and M. Kuchenreuther, unpublished data) have shown that *G. richardsonii* does self and suffers inbreeding depression in fruit and seed set. Although autogamy appears rare in *G. richardsonii*, because of its large, asynchronous floral display, geitonogamy may contribute significantly to selfing in this species.

We estimated the proportion of pollen received from geitonogamy as the difference in pollen receipt on stigmas of emasculated target flowers on emasculated (E) and unemasculated (U) plants in the geitonogamy experiment. In 1997 there was 19% and in 1998 42% more pollen received by flowers on unemasculated plants. Therefore, although hermaphrodites do receive over two times more pollen than females (see above), most is geitonogamous self-pollen. The percentage of the total difference in pollen receipt between hermaphrodites and females due to geitonogamy (U − E / C − F) was 66% in 1997 and 93% in 1998. The greater pollen receipt by hermaphrodites relative to females results primarily from geitonogamous self-pollination and to a lesser extent from higher pollinator attraction and outcrossing to their larger flowers. The relative fitness of hermaphrodites and females resulting from these differences depends on how post-pollination events, including inbreeding depression, affect the outcome of self-pollinations. Given the large standard errors surrounding counts of pollen receipt,

these figures only provide crude estimates of levels of autogamy and geitonogamy. They do suggest that selfing, primarily through geitonogamy, occurs at magnitudes sufficient to depress hermaphrodite fitness if selfing results in inbreeding depression. These estimates also appear to reflect actual levels of selfing occurring in the field. Genetic estimates of selfing rates, including geitonogamy, in open-pollinated hermaphrodites (Ritland and Jain, 1981) from four populations of *G. richardsonii* at RMBL ranged from 51 to 67% (mean = 61%) (K. O'Malley, C. Williams, and M. Kuchenreuther, unpublished data). Agren and Willson (1991) estimated that the selfing rates in two populations of *Geranium maculatum* were 60 and 73%, based on inbreeding depression. Similarly high selfing rates have been found in hermaphrodites of other gynodioecious species (23–25% in *Limnanthes douglasii*—Kesseli and Jain, 1984; 11–76% in *Bidens*—Sun and Ganders, 1986; 18–61% in *Salvia pratensis,* Van Treuren et al., 1993; 88–100% in *Chionographis japonica*—Maki, 1993;. 73% in *Cucurbita foetidissima*—Kohn and Biardi, 1995; 68% in *Schiedea adamantis*—Sakai et al., 1997).

A puzzling result of the pollination experiments were the sometimes smaller estimates of pollen receipt on stigmas of unemasculated flowers than on emasculated flowers on unemasculated plants (U vs. E in autogamy experiment; C vs. U in geitonogamy experiment). A possible explanation for these results may be that the stamens, particularly the filaments, may act as a physical barrier to small pollinators contacting the stigmatic surface. By removing these interfering structures by emasculation, access to the stigmas and pollen deposition might be enhanced. More careful observations of pollinator behavior are needed to address this possibility.

Density- and frequency dependent pollination success—Both resource allocation and avoidance of selfing appear to favor females in these populations of *G. richardsonii*. Female frequencies ranged from 0 to 83% of flowering plants in different populations throughout the flowering season. Given this wide range of female frequency and density, the relative fitness of females and hermaphrodites may vary under different demographic circumstances. Only a weak relationship between density of flowering hermaphrodites and pollination success was found. However, a strong negative relationship between relative female pollination success and female frequency in populations was observed. Such negative frequency dependence should set an upper limit on female frequency in populations because as females become more common they will suffer from pollen limitation. McCauley and Brock (1998) found that various measures of female and hermaphrodite reproductive success were frequency dependent in gynodioecious *Silene vulgaris*. Sun and Ganders (1986) and Van Treuren et al. (1993) also found that selfing rates in hermaphrodites were positively correlated with female frequency in populations of *Bidens* and *Salvia,* respectively. Frequency-dependent fitness and local population genetic structure can maintain gynodioecy as a stable polymorphism in a metapopulation (McCauley and Taylor, 1997).

Conclusions—In this study we have identified several

prerequisites for the selective maintenance of gynodioecy in populations of *Geranium richardsonii.* Females produce significantly smaller flowers than hermaphrodites. This floral dimorphism may allow females to allocate more resources to seed production than hermaphrodites. Although females produce 1.2 times more seeds per flower than hermaphrodites (C. Williams, unpublished data), long-term studies are underway to better elucidate fecundity differences in this long-lived perennial. Floral dimorphism is also associated with two other measures of reproductive success, pollinator attraction and pollen receipt. Females receive fewer pollinator visits and less pollen on their stigmas than hermaphrodites. Most of this difference appears to result from geitonogamy. Self-pollination through geitonogamy in hermaphrodites sets up the potential for females to gain a fitness advantage over hermaphrodites if such selfing results in inbreeding depression. Further studies of the magnitude of inbreeding depression on seed production and progeny fitness are in progress to determine how differences in pollination affect the relative fitness of females and hermaphrodites in this species. In addition, since the relative success of females at receiving pollen declines as their frequency in the population increases, selection opposing females may occur at high female frequencies due to pollen limitation. Understanding the maintenance of gynodioecy in *G. richardsonii* will depend on quantifying patterns of resource allocation, selfing rates, inbreeding depression, and pollen limitation in the context of the frequency of floral morphs. These studies are currently underway in natural populations of *Geranium richardsonii* spanning a wide range of floral morph frequencies.

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