

POLLINATION, BREEDING SYSTEM, AND GENETIC STRUCTURE IN TWO SYMPATRIC *DELPHINIUM* (RANUNCULACEAE) SPECIES¹

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Two sympatric *Delphinium* species, *D. barbeyi* and *D. nuttallianum*, are ecologically and morphologically similar. However, *D. barbeyi* has multiple, large inflorescences while *D. nuttallianum* has a single, small inflorescence. These differences in floral display should result in greater intraplant pollen transfer in *D. barbeyi*, leading to higher rates of self-pollination through geitonogamy. Reduced gene flow by pollen should in turn produce greater population differentiation among populations of *D. barbeyi* relative to *D. nuttallianum*. We tested these predictions by comparing pollinator behavior, breeding systems, outcrossing rates, and population genetic structure of sympatric populations of the two species in Colorado. Bumble bee and hummingbird pollinators visit more flowers and inflorescences per foraging bout in *D. barbeyi* than in *D. nuttallianum*. The species differed in breeding system; *D. barbeyi* produced more seeds by autogamy (9 vs. 2%) than *D. nuttallianum* and suffered no reduction in seed set in hand-self vs. outcross pollinations, in contrast to a 41% decline in *D. nuttallianum*. The outcrossing rate in one *D. barbeyi* population was 55%, but ranged from 87 to 97% in four *D. nuttallianum* populations. Genetic differentiation among population subdivisions estimated by hierarchical *F* statistics was >10 times greater in *D. barbeyi* ($\hat{\theta} = 0.055\text{--}0.126$) than *D. nuttallianum* ($\hat{\theta} = 0.004\text{--}0.009$) at spatial scales ranging from metres to 3.5 km. Spatial autocorrelation analysis also indicated more pronounced local genetic structure in *D. barbeyi* than *D. nuttallianum* populations. Fixation indices (F_{IS}) of *D. barbeyi* adults were much lower than expected based on mating system equilibrium and suggest that differences in the degree of self-compatibility and/or the timing of postpollination selection/inbreeding depression between the two species further contribute to the genetic differences between them.

Key words: breeding system; comparative study; floral display size; *F* statistics; geitonogamy; genetic structure; outcrossing rate; pollinator behavior.

Pollinator behavior and plant mating systems are influenced by a variety of plant traits, including floral morphology and phenology, self-incompatibility, and inflorescence architecture (Wyatt, 1982; Richards, 1986; Harder and Barrett, 1996). In turn, the mating system is a primary determinant of plant population genetic structure. Inbreeding species are expected to have less genetic diversity and heterozygosity within populations and more genetic differentiation among populations than outcrossing species (Wright, 1921, 1951; Allard, Jain, and Workman, 1968; Jain, 1976). Surveys comparing allozyme and quantitative genetic variation across a wide range of taxa generally support these theoretical predictions (Brown, 1979; Hamrick, Linhart, and Mitton, 1979; Schoen and Brown, 1991; Charlesworth and Charlesworth, 1995; Hamrick and Godt, 1996). However, interspecific comparisons of many unrelated taxa may be confounded by the effects of correlated ecological traits or phylogeny in producing the observed pattern of genetic structure. In addition, comparisons of rare vs. widespread

species indicate that measures of genetic diversity are often strongly correlated between congeneric species (Gitzendanner and Soltis, 2000). Comparative studies of closely related taxa have the advantage of being able to better isolate the effects of variation in single traits. To this end, numerous intrageneric and intraspecific comparisons have examined the relationship between the mating system and allozyme diversity and heterozygosity within populations. Most of these studies support the expected reduction in diversity and heterozygosity of selfers relative to outcrossers (reviewed by Brown, 1979; Schoen and Brown, 1991; Charlesworth, Nordborg, and Charlesworth, 1997).

In contrast, relatively few studies of closely related taxa have examined the effects of mating system variation on genetic differentiation among population subdivisions (Appendix). In most cases, these studies found that species with more highly selfing mating systems had greater inbreeding within subpopulations and more genetic differentiation among subpopulations. These studies mostly compared taxa with widely divergent mating systems (e.g., selfing vs. outcrossing or self-compatible vs. self-incompatible) and/or taxa that differ in flower traits affecting the breeding system (e.g., flower size, degree of protandry). Floral display, the number and arrangement of flowers on the plant, can also affect the mating system (Wyatt, 1982; Geber, 1985; Harder and Barrett, 1996; Snow et al., 1996). In particular, the number of open flowers on the plant should increase pollinator attraction, but is predicted to lead to more selfing through geitonogamy (the transfer of self pollen between flowers on the same plant) (Hessing, 1988; de

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Jong, Waser, and Klinkhamer, 1993; Harder and Barrett, 1996; Snow et al., 1996). These potentially conflicting effects of floral display size have been little studied in regard to their influence on the mating system and gene flow.

The purpose of our study was to investigate differences in floral display (plant size and number of open flowers) and the breeding system (autogamy and self-compatibility) and their effects on pollinator behavior, outcrossing rates, and population genetic structure in sympatric *Delphinium barbeyi* Huth and *Delphinium nuttallianum* Pritzel (= *D. nelsonii* Greene) (Ranunculaceae). These species share most life-history traits thought to affect genetic structure. Both are long-lived herbaceous perennials. They co-occur in the same subalpine meadows, with *D. nuttallianum* found on somewhat drier microsites. *Delphinium barbeyi* has limited clonal spread, forming distinct clumps, and *D. nuttallianum* has no vegetative reproduction. Both have gravity-dispersed seeds. The two species have morphologically similar, protandrous flowers (*D. barbeyi* flowers are slightly smaller). Although *D. nuttallianum* blooms earlier in the season, the species have similar flower visitors. The main pollinators of *Delphinium barbeyi* are hummingbirds (*Selasphorus platycercus*, *S. rufus*, and *Stellula calliope*), and queen and worker bumble bees (especially *Bombus appositus* and *B. flavifrons*). *Delphinium nuttallianum* is visited more by *S. platycercus* and queen bees (Waser, 1982). *Delphinium* is a predominantly outcrossing but self-compatible genus (Epling and Lewis, 1952), with widely varying degrees of self-incompatibility (0–50%) and autogamy (1–90%) reported among species (Macior, 1975; Varney, 1979; Powell and Jones, 1983; Bosch, 1999). Autogamy rates, partial self-incompatibility, and inbreeding depression have been well documented in *D. nuttallianum* (Waser, 1978; Price and Waser, 1979; Waser and Price, 1991, 1994). Comparable data on the breeding system of *D. barbeyi* are presented here.

The primary morphological difference between the two species is the size of their floral display. *Delphinium barbeyi* produces hundreds of flowers per genet, usually on multiple stalks, whereas *D. nuttallianum* produces only a few flowers on a single raceme (Waser, 1982). Though pollinators fly similar distances between flowers in the two species (Waser, 1982), the higher density of genetically distinct individuals in *D. nuttallianum* means that pollen travels to a greater number of genets. Conversely, *D. barbeyi* potentially receives more self pollen by virtue of the greater proportion of intraplant pollinator flights (*D. barbeyi*, 67.4%, $N = 954$ visits; *D. nuttallianum*, 44.8%, $N = 2336$ visits; $\chi^2 = 138.77$, $df = 1$, $P < 0.0001$; reanalyzed data of Waser, 1982). These differences in pollen dispersal should lead to more inbreeding and less gene flow (Crawford, 1984) in *D. barbeyi* than in *D. nuttallianum*. We therefore expect a lower outcrossing rate (t), a higher inbreeding coefficient (f or F_{IS}), and a greater degree of population subdivision (higher θ or F_{ST}) in many-flowered *D. barbeyi* than in few-flowered *D. nuttallianum*. We report here comparisons of floral display size, pollinator foraging behavior, breeding system, outcrossing rates estimated from progeny arrays, and genetic structure over a variety of spatial scales for sympatric populations of *D. barbeyi* and *D. nuttallianum*.

MATERIALS AND METHODS

Study sites—We studied *D. barbeyi* and *D. nuttallianum* from 1990 to 1995 in subalpine meadows near the Rocky Mountain Biological Laboratory

(RMBL, 2900 m elevation) in southwestern Colorado, USA. Physical and floristic characteristics of the site are described elsewhere (Langenheim, 1962; Bosch and Waser, 1999). *Delphinium nuttallianum* begins flowering shortly following snowmelt in late May or early June. *Delphinium barbeyi* typically begins blooming in late June or early July, at the end of the flowering period of *D. nuttallianum* (Waser, 1978). All observations and experiments were carried out in *Delphinium* populations along a 3.5-km corridor of the East River valley and its tributary Copper Creek.

Floral display size—The number of flowers per inflorescence and number of inflorescences (flowering stems) per plant were examined on 61 *D. barbeyi* plants in the Gothic Research Meadow in 1990. Additionally, 17 plants in 1990 and 12 plants in 1992 were monitored throughout the flowering period to estimate the time of peak flowering and the mean number of flowers open per day. The number of flowers and inflorescences per plant were estimated for *D. nuttallianum* in four populations in 1991. The number of open flowers in the male phase (during anther dehiscence) and female phase (during stigma receptivity) were counted for each plant.

Pollinator behavior at *Delphinium barbeyi*—*Paired plant experiment*—We observed visitation by bumble bees to paired large and small plants in the Gothic Research Meadow. Per-plant and per-flower visit rates to two neighboring plants differing in size were recorded in seven 30-min trials between 1430 and 1730 MDT on 5–9 August 1990. Because the distributions of visitation measures were highly skewed, the central tendencies are presented as medians and midranges (middle 50% of observations) in this and the following analysis. Differences between large and small plants in visitation measures were analyzed using Wilcoxon matched-pairs signed ranks tests (Sokal and Rohlf, 1981).

Individual foraging bouts—Foraging bouts of individual bumble bees and hummingbirds were observed in a patch of 60 marked *D. barbeyi* plants between 28 July and 5 August 1990. We followed bumble bees foraging within the patch, placed a numbered flag by each plant visited, and recorded the number of flowers visited. We subsequently counted the number of open flowers on each visited plant. Hummingbirds were observed with binoculars from a distance of 10–25 m as they visited plants marked with numbered flags. For each bout we recorded the number of flowers visited on flagged, numbered plants. We counted the number of open flowers on marked plants each day. For bees and hummingbirds, we analyzed the relationship between plant size and flower visitation frequency using linear regression (Sokal and Rohlf, 1981).

Inflorescence number—We also examined the effect of *D. barbeyi* plant size on bee foraging behavior by classifying plant size as the number of inflorescences with at least two open flowers. Plants were divided into 6 different size classes (1, 2–5, 6–10, 11–15, 16–24, and >24 inflorescences). Individual plants were observed for 10 min each, between 1100 and 1800 on 22–26 July 1994. No plant was observed more than twice. We recorded the number of bee visits to a plant per 10-min observation period and the number of different inflorescences visited during each bee's foraging bout. The relationships between the response variables (bee visits and number of inflorescences visited) and plant size were analyzed using one-way ANOVA (Sokal and Rohlf, 1981). Plants were further grouped into small (≤ 10 inflorescences) and large (> 10 inflorescences) size classes and differences in foraging between these two groups were analyzed using ANOVA.

Breeding system—We investigated the breeding system of *D. barbeyi* using five different pollination treatments. Each treatment was performed on a separate inflorescence and replicated on eight different plants. At least nine flowers on each plant received each treatment. To test for autogamy, flowers were left unemasculated and the inflorescence covered with a fine mesh bag to exclude pollinators. To compare self and outcross pollination, inflorescences were kept covered and new male-phase flowers were emasculated each day. Pollen was applied daily to receptive stigmas using toothpicks. We hand-pollinated each flower for three consecutive days to assure female receptivity.

For the hand-self treatment the pollen source was another flower from the same plant. For the hand-outcross treatment flowers from plants growing at least 10 m away served as donors. We marked two additional inflorescences on each plant, which were left open (unbagged) to pollinator visits. Flowers on one open inflorescence were unmanipulated and served as an open-pollinated control (natural treatment). Flowers on the other open-pollinated inflorescence were also hand-outcrossed daily with supplemental pollen to test for pollen limitation. We performed pollination treatments from 24 July to 6 August 1990 and collected fruits to assess seed set on 13 August. Because not all treatments were successful on each plant, we used paired *t* tests to compare seed set per flower between paired treatments on each plant. We compare these results to those published for *D. nuttallianum* (Waser, 1978; Waser and Price, 1991).

Genetic techniques—We used allozymes as genetic markers to estimate the mating system and genetic structure for both species. Horizontal starch-gel electrophoresis was performed on both leaf material and seeds. The electrophoretic techniques and grinding and running buffers for leaves and seeds of both species were as described in Williams and Waser (1999) for *D. nuttallianum* leaves. In *D. barbeyi*, we resolved three polymorphic isozymes in both leaf and seed material: phosphoglucose isomerase (*Pgi-2*; three alleles), phosphoglucosmutase (*Pgm-1*; three alleles), and acid phosphatase (*Acp-2*; four alleles). Allozyme results from leaf material used for analysis of genetic structure in *D. nuttallianum* are reported in Williams and Waser (1999). In *D. nuttallianum* we resolved four polymorphic isozymes from seeds: malate dehydrogenase (*Mdh-1*, two alleles; *Mdh-2*, three alleles), phosphoglucosmutase (*Pgm-1*; three alleles), and phosphoglucose isomerase (*Pgi-2*, three alleles). These loci yielded clear banding patterns consistent with their previously reported quaternary structure (Kephart, 1990) and segregated in expected Mendelian fashion in progeny arrays.

Outcrossing rates—We estimated the mating system of each species from allozyme genotypes of seeds in progeny arrays. Fruits of *D. nuttallianum* were collected from two populations at each of two sites (populations KPA, KPB, JFA, and JFB of Williams and Waser, 1999) in 1991. Fruits were air dried and stored at room temperature in coin envelopes for 12–18 mo before using seeds for electrophoresis. *Delphinium barbeyi* seed collected for mating system analysis in 1995–1996 were lost because of insect predation. We successfully collected mature infructescences from 22 *D. barbeyi* plants in the KPB population in late August 1997. Infructescences were first oven dried at 50°C for several days to arrest fungal growth and destroy the eggs and larvae of a dipteran seed predator. We stored fruits at room temperature with a small amount of para-dichlorobenzene until using the seeds for electrophoresis in January 1998.

We estimated the outcrossing rates of both *Delphinium* species using the multilocus maximum-likelihood, mixed-mating procedure of Ritland and Jain (1981) with the computer program MLT written by K. Ritland. All seeds from a single maternal plant (from 1 to 5 fruits) were treated as separate families or progeny arrays. The maternal genotypes of most *D. nuttallianum* plants were known from separate analysis of leaf material. We inferred maternal genotypes of all *D. barbeyi* and a few *D. nuttallianum* plants from progeny arrays using the procedure of Brown and Allard (1970), implemented in MLT. In the analysis, pollen and ovule genotype frequencies were estimated separately. The standard errors (SE) of multilocus outcrossing rates were calculated from the distribution of 500 bootstrap estimates, where the unit of resampling was the family (progeny array of a maternal plant).

Spatial genetic structure—*Sampling design*—We used a nested, hierarchical sampling design to analyze the genetic structure of both species. In 1995 *Delphinium barbeyi* were sampled at four sites, each site containing three populations, and each population made up of three subpopulations. These four sites (KP, RM, CC, and BP) correspond to sympatric *D. nuttallianum* sites (Kettle Ponds, Research Meadow, Copper Creek, and 401 Trail, respectively) sampled in an earlier study (Williams and Waser, 1999). The four sites were isolated by forest or other habitat barriers unsuitable for *Delphinium* and were separated by ≥ 1 km. We located three semi-isolated populations, 40–120 m

apart (e.g., populations KPA, KPB, and KPC at site KP), which formed distinct patches within the larger metapopulation at each site. In each population we mapped all plants within 10 m of a transect through its center. Transects were further subdivided along their length in approximate thirds to create three subpopulations per population. Sample sizes per population ranged from 25 to 125 plants (total $N = 725$ mapped plants). A small amount of leaf material was collected from each mapped plant and refrigerated until used for electrophoresis, usually within 24 h.

We sampled *D. nuttallianum* at the same four sites used for *D. barbeyi*, plus two additional sites within the East River valley. Each of the six sites contained two (three at one site) populations, and each population was composed of four subpopulations. Sample sizes ranged from 40 to 340 individuals per population (total $N = 1620$ plants). Further details of the sampling design and a map of sampled sites for *D. nuttallianum* are described elsewhere (Williams and Waser, 1999).

F statistics—We estimated hierarchical *F* statistics using the approach of Weir and Cockerham (1984), in which total genetic variation, F_{IT} , is apportioned into components due to differentiation among nested population subdivisions, θ [equivalent to Wright's (1951) F_{ST}] and that due to fixation within the smallest subdivisions, F_{IS} . We calculated genotype frequencies and estimated genetic differentiation among three nested levels of population subdivision. These three estimators are: (1) $\hat{\theta}_{SUBPOP}$, differentiation among subpopulations within populations, (2) $\hat{\theta}_{POP}$, differentiation among populations within sites, and (3) $\hat{\theta}_{SITE}$, differentiation among sites within the total sample. Comparable hierarchical results for *D. nuttallianum* are summarized from an earlier paper (Williams and Waser, 1999). The 95% confidence intervals (CIs) of the multilocus estimates of each *F* statistic were calculated from the distribution of 1500 bootstraps over loci (Weir and Cockerham, 1984). We tested deviations of $\hat{\theta}$ from zero and differences between species' estimates by nonoverlap of the 95% confidence intervals of multilocus estimates.

Spatial autocorrelation—Analyses of spatial autocorrelation between individual plants on single-locus allele frequency data were performed using a computer program provided by J. S. Heywood. The autocorrelation coefficient, Moran's *I*, was calculated for each allele between all nearest-neighbor pairs of individuals (I_{NN}) in each of five *D. barbeyi* populations containing >75 mapped plants and in four mapped *D. nuttallianum* populations (Williams and Waser, 1999). Autocorrelation coefficients were also calculated among all pairs of individuals in 1-m distance intervals over 0–20 m in these populations. We considered single allele estimates of autocorrelation coefficients significantly different from random expectations at $P < 0.05$ (two-tailed) if the standard normal deviate (SND) of the estimate was ≥ 1.96 , where $SND = [I - \mu]/\sigma$ (Heywood, 1991). We calculated the mean autocorrelation coefficients for nearest neighbors and at 1-m intervals for each population by averaging single-allele estimates in each distance class. The least common allele at each locus was omitted from the averages to maintain statistical independence of samples.

RESULTS

Floral display—The number of flowers produced per flowering stem (= inflorescence) and per plant is much greater in *D. barbeyi* than in *D. nuttallianum* (Table 1). *Delphinium barbeyi* plants are typically multistemmed, producing an average of >20 flowers per stem. The number of flowering stems per plant varies widely, averaging 16 stems per plant. The total number of flowers produced per plant therefore averages almost 450. In 1992 the flowering period extended for 42 d (1 July–11 August) with a duration of flowering of 28.2 ± 5.8 d/plant (mean \pm SD). Peak flowering (maximum number of open flowers) occurred 16.1 ± 4.4 d into a plant's flowering period. An average of ~ 170 flowers per plant were open at peak bloom (Table 1).

Delphinium nuttallianum plants produce a single flowering

TABLE 1. Floral display size and flowering behavior in one population of *Delphinium barbeyi* in two years, and four *D. nuttallianum* populations in a single year near the RMBL.

<i>D. barbeyi</i>								
Year	N	Stems per plant		Flowers per stem	Flowers per plant		Flowers open per day ^a	
		$\bar{X} \pm SD$	Range	$\bar{X} \pm SD$	$\bar{X} \pm SD$	Range	$\bar{X} \pm SD$	Range
1990	61 ^b	12.2 ± 9.0	1–43	22.8 ± 9.7	302 ± 336	43–1746	168 ± 150	32–513
1992	12	20.1 ± 11.5	5–41	27.9 ± 7.9	582 ± 423	83–1430	177 ± 141	15–463
<i>D. nuttallianum</i>								
Year	N	Stems per plant		Flowers per plant		Flowers open per day ($\bar{X} \pm SD$)		
		$\bar{X} \pm SD$	Range	$\bar{X} \pm SD$	Range	Male	Female	Total
1991	1240	1.02 ± 0.14	4.67 ± 2.93	1–31	2.14 ± 1.62	0.95 ± 0.93	3.09 ± 1.87	0–13

^a Highest count for each plant.

^b Sample size (N) for “flowers open per day” was 17 plants in 1990; for other variables, N = 61 plants.

stem with occasional small lateral flowering branches (Table 1). The length of the flowering period is ~20–30 d (F. Saavedra and C. Williams, unpublished data), beginning soon after snowmelt in late May or early June. In 1991 the total number of flowers per plant averaged 4.7 flowers in four populations. The average number of open flowers per plant was 3.1, 2.1 in male phase and 1.0 in female phase (Table 1).

Pollinator behavior—Paired plant experiment—A total of 2965 visits by bumble bees to *D. barbeyi* flowers were recorded in seven 30-min observations of paired large and small plants in 1990. The larger plants had significantly more bumble bee approaches, a significantly greater median number of flowers visited, and significantly higher per flower visitation rates than the small plants (Table 2). Bees often visited a relatively large number of flowers per plant, especially on larger plants (Table 2). Workers of *Bombus appositus* and *B. flavifrons* accounted for 80 and 19%, respectively, of flower visits, with the remainder by queen *B. appositus*.

Individual foraging bouts—Bumble bee workers and rufous and calliope hummingbirds followed from plant to plant during foraging bouts in 1990 showed the same tendency to visit more flowers on larger plants. Linear regressions of flower visits on plant size were significant for all three visitor taxa, with the proportion of variance explained by plant size ranging from 0.13 in rufous hummingbird to 0.28 in bumble bees (Table 3). Visitation patterns provided opportunities both for out-

crossing and geitonogamy. The median number of flowers visited per plant ranged from 7 to 14, although some very lengthy bouts were observed. The median number of flowers visited was low relative to the number of flowers open per plant, ranging from 8 to 13% (Table 3). Calliope hummingbirds had longer foraging bouts than territorial rufous hummingbirds, despite the fact that 47% of their foraging bouts ended with a chase by the territorial species. However, the two species visited similar numbers of flowers per plant.

Inflorescence number—Bumble bees again showed a pattern of higher visitation rates to larger plants when inflorescence number was used as the size criterion. Bees, including queen and worker *Bombus appositus* and *B. flavifrons*, visited larger plants more frequently than small plants during 61 10-min observation periods ($F_{5,55} = 3.41$, $P = 0.009$; Table 4). When plants are grouped into large (>10 inflorescences) and small (≤ 10 inflorescences), the number of bee visits per 10 min was significantly greater for large than for small plants ($F_{1,59} = 15.56$, $P = 0.0002$; Table 4). Bees also visited multiple inflorescences on the same *D. barbeyi* plant during a foraging bout. Although there were no significant differences in number of inflorescences visited among the six size classes ($F_{5,81} = 1.71$, $P = 0.142$), when plants are grouped into large and small size classes as above, bees visited significantly more inflorescences per bout on large plants than small plants ($F_{1,85} = 6.82$, $P = 0.011$; Table 4).

TABLE 2. Patterns of bumble bee (*Bombus appositus* and *B. flavifrons*) visitation to paired large and small *Delphinium barbeyi* plants. Plant size is number of open flowers. Results of matched-pairs signed-rank tests for each visitation measure are given.

Trial no.	Plant size (large : small)	Visitation rate (per 30-min trial)		
		Approaches per bout (large : small)	Flowers per plant: median (midrange ^b) (large : small)	Visits per flower per trial (large : small)
1	423 : 82	12 : 7	32 (21–46) : 2 (1–6)	1.00 : 0.40
2	150 : 88	12 : 5	12 (5–27) : 8 (8–11)	1.57 : 0.51
3	221 : 54	10 : 3	22 (1–50) : 12 (1–13)	1.40 : 0.48
4	139 : 31	11 : 5	9 (5–27) : 2 (1–3)	1.19 : 0.39
5	513 : 150	29 : 23	28 (19–32) : 6 (3–13)	1.61 : 1.39
6	327 : 144	26 : 7	13 (6–28) : 15 (3–54)	1.43 : 1.21
7	296 : 163	33 : 14	18 (7–26) : 9 (3–15)	2.17 : 1.21
Difference: ^b median (midrange)		7 (6–19)	9.5 (4–22)	0.81 (0.22–0.96)
Probability ^c		<0.05	<0.05	<0.05

^a Range for the middle 50% of values.

^b Difference between large and small plants in the visitation measure was calculated as: value for large plants minus value for small plants. The median value of this difference is given (N = 7 trials), followed by the midrange. The null hypothesis predicts a median difference of zero. For flowers per bout, the difference for each trial was calculated as: median for large plants minus median for small plants.

^c Wilcoxon matched-pairs signed-ranks test.

TABLE 3. Patterns of plant and flower visitation during foraging bouts by bumble bee workers and rufous and calliope hummingbirds and linear regressions of flower visits on plant size for each visitor type. Observations were made between 27 July and 4 August 1990. Summary statistics for the first four characteristics are reported as median (midrange, maximum); midrange includes the middle 50% of values.

Characteristic	Bumble bee workers	Rufous hummingbird	Calliope hummingbird
No. plants visited/bout ^a	3 (1–4, 8)	2 (1–2, 6)	6 (3–9, 11)
No. flowers visited/bout ^a	39 (11–62, 211)	23 (12–43, 113)	142 (85–175, 283)
No. flowers visited/plant	7 (2–17, 127)	12 (5–21, 68)	14 (6–27, 138)
No. open flowers/plant	91 (48–192, 526)	92 (46–192, 526)	170 (94–250, 526)
Regression equation ^b	0.079X + 2.7	0.038X + 9.9	0.096X + 3.4
Regression r^2	0.28	0.13	0.21
Bouts observed	46	58	17

^a Partial bouts of bumble bee workers (*Bombus appositus*, *B. flavifrons*); complete bouts of hummingbirds.

^b Linear regression equation: $y = bX + a$, where y = flowers visited/plant, X = open flowers/plant, and a = intercept. All regressions were significant ($P < 0.001$ that the regression slope equals zero).

Breeding system—*Delphinium barbeyi* produces significantly fewer seeds by autogamy than open pollination (paired $t = 2.80$, $df = 6$, $P < 0.05$), hand-outcross pollination (paired $t = 5.87$, $df = 6$, $P < 0.01$), or hand-self pollination (paired $t = 3.47$, $df = 6$, $P < 0.05$). Flowers in the autogamy treatment produced only 9.2, 16.1, and 13.6% as many seeds as flowers in the natural, hand-outcross, and hand-self treatments, respectively (Table 5). *Delphinium barbeyi* experienced no reduction in seed set in hand-self vs. hand-outcross pollinations when treatment means for six matched plants were compared (hand-self: 7.65 ± 1.54 seeds/flower; hand-outcross: 7.62 ± 1.52 seeds/flower; paired $t = 0.02$, $df = 5$, $P > 0.9$); however, plants varied in their responses, with three plants showing at least a twofold difference between hand-self and hand-outcross treatments. Addition of supplemental pollen to open-pollinated flowers produced higher seed set in only half of the six plants with matched treatments and was not significant (paired $t = 1.04$, $df = 5$, $P > 0.20$).

By comparison, an earlier experiment on *D. nuttallianum* (Waser, 1978) showed that autogamy was rare, seed set of bagged flowers averaging only 2.2% of open-pollinated flowers (Table 5). Likewise, three previous experiments (Waser and Price, 1991) demonstrated that self-pollinated flowers produced an average of only 59.4% as many seeds as outcrossed matings between plants growing 10 m apart (Table 5).

Outcrossing rates—The multilocus outcrossing rate estimate (t_m) for *D. barbeyi* was significantly lower than those of *D. nuttallianum* (Table 6). Multilocus estimates were signifi-

cantly < 1.0 in *D. barbeyi* and *D. nuttallianum* populations KPA and KPB. The selfing rate ($1 - t_m$) in *D. barbeyi* was almost 45%, indicating a mixed-mating system. In contrast, the selfing rate averaged only 7% in the four *D. nuttallianum* populations. Differences between average single-locus (t_s) and multilocus (t_m) outcrossing rates, an estimate of the contribution of biparental inbreeding to estimated selfing, are small and not significant.

F statistics—Genetic differentiation among population subdivisions was significantly greater in *D. barbeyi* than *D. nuttallianum* (Table 7). At each level of the spatial hierarchy, estimates of θ were > 10 times larger, suggesting much more restricted gene flow in *D. barbeyi*. There was significantly greater genetic differentiation at the two larger scales than at the smallest scale (among subpopulations within populations) for *D. barbeyi*. In *D. nuttallianum* there were no significant differences in the degree of genetic differentiation among different levels of the spatial hierarchy. Both \hat{F}_{IT} and \hat{F}_{IS} differed significantly between the two species. \hat{F}_{IT} was greater in *D. barbeyi* as expected. However, \hat{F}_{IS} was significantly smaller in *D. barbeyi* than in *D. nuttallianum* and significantly < 0 , indicating an unexpected heterozygote excess in adults of the apparently more inbred species.

Spatial autocorrelation—Spatial autocorrelation analysis revealed more fine-scale spatial genetic structure in *D. barbeyi* than in its congener *D. nuttallianum*. Average near-neighbor autocorrelation coefficients were higher with more significant

TABLE 4. Bee foraging behavior in relation to *Delphinium barbeyi* plant size (number of inflorescences) in 1994. Each plant was observed for 10 min.

Plant size	No. bee visits (bouts) per 10 min			No. inflorescences visited per bout		
	N ^a	$\bar{X} \pm SE$	Range	N ^a	$\bar{X} \pm SE$	Range
1	11	0.36 ± 0.15	0–1	4	1.00 ± 0.00	1
2–5	10	0.90 ± 0.28	0–2	9	2.8 ± 0.86	1–9 ^c
6–10	10	0.90 ± 0.23	0–2	9	2.78 ± 0.62	1–6
11–15	10	2.40 ± 0.79	0–9	24	4.17 ± 0.44	1–7
16–24	10	2.00 ± 0.62	0–7	20	5.10 ± 0.81	1–14
>24	10	2.90 ± 0.82	0–8	21 ^d	4.62 ± 1.03	1–20
Small (1–10)	31	0.71 ± 0.13	0–2	22	2.50 ± 0.45	1–9
Large (>10)	30	2.43 ± 0.42	0–9	65	4.60 ± 0.44	1–20

^a Number of observation periods per size class.

^b Total number of foraging bouts observed per size class.

^c Includes repeat visits to same inflorescence.

^d Data missing for eight bouts from one plant.

TABLE 5. Seed set under different pollination treatments in *Delphinium barbeyi* and *D. nuttallianum*. Data for *D. barbeyi* are per flower means \pm 1 SE (*N*) from experiments conducted on eight plants in 1990 at the RMBL, except that values in the final row are per plant means. Data for *D. nuttallianum* are per flower means \pm 1 SE (*N*) from three experiments previously reported by Waser (1978) and Waser and Price (1991).

<i>D. barbeyi</i> (1990) Plant	Pollination treatment				
	Autogamy	Hand-self	Hand-outcross	Natural	Supplemental
1	0.0 \pm 0.0 (26)	7.6 \pm 1.5 (26)	11.0 \pm 2.5 (26)	11.1 \pm 2.9 (24)	23.7 \pm 3.8 (18)
2	0.0 \pm 0.0 (16)	6.8 \pm 1.5 (11)	4.8 \pm 2.4 (10)	13.1 \pm 4.6 (12)	38.3 \pm 1.1 (12)
3	2.8 \pm 1.2 (14)	4.1 \pm 1.5 (16)	8.0 \pm 2.5 (23)	3.4 \pm 2.6 (12)	28.6 \pm 2.7 (16)
4	4.8 \pm 1.5 (16)	12.0 \pm 1.6 (23)	12.3 \pm 1.3 (32)	24.9 \pm 3.3 (23)	17.5 \pm 3.9 (20)
5	0.0 \pm 0.0 (24)	12.1 \pm 1.8 (19)	2.4 \pm 1.3 (13)	6.8 \pm 2.5 (14)	5.4 \pm 2.6 (19)
6	1.0 \pm 0.5 (23)	21.2 \pm 2.3 (17)	—	33.7 \pm 1.3 (24)	21.3 \pm 4.8 (12)
7	0.0 \pm 0.0 (19)	—	4.7 \pm 0.9 (13)	0.5 \pm 0.4 (20)	—
8	0.5 \pm 0.3 (14)	3.3 \pm 2.0 (9)	7.2 \pm 1.6 (20)	—	3.4 \pm 1.9 (11)
Means per plant ^a	1.1 \pm 0.6 (8)	9.6 \pm 2.3 (7)	7.2 \pm 3.6 (7)	13.4 \pm 4.5 (7)	19.7 \pm 4.7 (7)

Year	Pollination treatment				Reference
	Autogamy	Hand-self	Hand-outcross ^b	Natural	
1975	1.1 \pm 0.51 (28)	—	—	49.5 \pm 2.35 (28)	Waser 1978
1976	—	12.3 \pm 1.8 (9)	14.8 \pm 2.4 (10)	—	Waser and Price 1991
1977a	—	5.2 \pm 1.2 (17)	11.9 \pm 2.3 (17)	—	Waser and Price 1991
1977b	—	9.0 \pm 2.5 (5)	14.9 \pm 2.6 (5)	—	Waser and Price 1991

^a See text for appropriate means for paired treatment comparisons, which differ from those listed here because of missing treatments in plants 6–8.

^b Seed set for 10-m interplant outcrossing distances (highest for each experiment) are shown.

single allele estimates in *D. barbeyi* populations than in three of the four *D. nuttallianum* populations. *D. barbeyi* exhibited a greater number of significant autocorrelation coefficients of larger magnitude over a greater distance than did *D. nuttallianum* (Table 8). In general, the pattern of spatial autocorrelation for most alleles was of decreasing positive coefficients over the first 2–6 m in all *D. barbeyi* populations. Thereafter autocorrelation coefficients fluctuated around zero with few significant values. In contrast, there was no pattern of local genetic similarity in three of the four *D. nuttallianum* populations (Table 8).

DISCUSSION

Floral display size and pollinator behavior—Our basic premise, that *D. barbeyi* plants present a much larger floral display and have more of their flowers visited per foraging bout by pollinators than *D. nuttallianum*, was supported by this study. The mean number of flowers open per day is almost two orders of magnitude higher in *D. barbeyi* than its sympatric congener *D. nuttallianum*. Such large differences in floral display size influence pollinators' behavior, which in turn influence the mating system and genetic structure of the plant

populations. The overall small daily number of open flowers (mean <4 open flowers) on the single inflorescence of *D. nuttallianum* should limit the possibilities for geitonogamy in this species. Bosch and Waser (1999) found that pollinators (mostly bees) visit an average of only 2.5 flowers per plant in *D. nuttallianum*. Furthermore, bumble bee movement among the strongly protandrous flowers of *D. nuttallianum* is characterized by bees arriving at older female-phase flowers low on the inflorescence and moving up, subsequently visiting younger male-phase flowers, promoting outcrossing (Pyke, 1978). There thus appears to be only limited opportunity for self-pollination, either by autogamy or geitonogamy, in this species.

Delphinium barbeyi on the other hand has an average of 170 flowers on multiple inflorescences open per day. Consequently, pollinators often visit many flowers per plant, and move between several inflorescences on the same plant during a foraging bout. Such interinflorescence movements are the most likely mechanism leading to self-pollination in *D. barbeyi*. More haphazard interflower and interplant movements by hummingbirds, which do not adhere as closely to the "bottom-up" foraging pattern of bumble bees (N. Waser, personal com-

TABLE 6. Multilocus (t_m), single-locus (t_s) outcrossing rates, and maternal fixation indices (F) for *Delphinium barbeyi* and *D. nuttallianum* estimated from progeny arrays. Standard errors (SE) of outcrossing rates are calculated from 500 bootstraps between families. Sample sizes (*N*) for number of plants per population, total seeds, and seeds per family (plant) are shown.

Species	Population	<i>N</i> plants	<i>N</i> seeds	Mean no. seeds/family	t_m (\pm SE)	t_s (\pm SE)	F (\pm SE)
<i>D. barbeyi</i>	KPB	22	499	22.2	0.554 (0.038)	0.513 (0.052)	0.000 (0.115)
<i>D. nuttallianum</i>	KPA	21	240	10.9	0.880 (0.045)	0.863 (0.062)	0.025 (0.157)
<i>D. nuttallianum</i>	KPB	59	865	14.7	0.916 (0.030)	0.885 (0.035)	0.117 (0.068)
<i>D. nuttallianum</i>	JFA	30	240	8.0	0.973 (0.076)	0.921 (0.068)	0.000 (0.121)
<i>D. nuttallianum</i>	JFB	20	159	8.0	0.935 (0.124)	0.997 (0.141)	0.090 (0.203)

TABLE 7. Single-locus and multilocus hierarchical F statistics for *Delphinium barbeyi* and comparable multilocus F statistics for *D. nuttallianum* (from Williams and Waser, 1999). See text for description of hierarchical sampling design for each species. Upper and lower 95% confidence limits (CL) of multilocus estimates (in parentheses) were calculated from the distribution of 1500 bootstrap estimates over loci. All multilocus estimates were significantly different between species ($P < 0.05$) as indicated by nonoverlap of their confidence limits.

Locus	\hat{F}_{IS}	$\hat{\theta}_{SUBPOP}$	$\hat{\theta}_{POP}$	$\hat{\theta}_{SITE}$	\hat{F}_{IT}
<i>D. barbeyi</i>					
<i>Pgi-2</i>	0.016	0.044	0.135	0.151	0.309
<i>Pgm-1</i>	-0.040	0.082	0.145	0.109	0.273
<i>Acp-2</i>	-0.067	0.038	0.094	0.118	0.179
Upper CL	(-0.002)	(0.069)	(0.142)	(0.141)	(0.298)
Multilocus estimate	-0.031	0.055	0.125	0.126	0.255
Lower CL	(-0.058)	(0.030)	(0.109)	(0.113)	(0.205)
<i>D. nuttallianum</i>					
Upper CL	(0.107)	(0.014)	(0.005)	(0.011)	(0.118)
Multilocus estimate	0.079	0.005	0.004	0.009	0.095
Lower CL	(0.042)	(-0.002)	(0.002)	(0.001)	(0.058)

munication), may further contribute to selfing in both species. Although the pollinator taxa are qualitatively similar between the two species, quantitative differences in pollinator abundance, pollen carryover, and seasonal variation in interspecific plant movements may also affect differences in the mating system between these *Delphinium* species. Because plant size increases attractiveness to pollinators in both species (e.g., Schulke, 1999 for *D. nuttallianum*), more detailed investigations of the effects of intraspecific variation in floral display on pollinator behavior and the mating system are needed.

Outcrossing rates and breeding systems—As predicted because of its larger floral display, *D. barbeyi* had a significantly greater selfing rate than *D. nuttallianum*. Pollinator foraging behavior in response to plant size and inflorescence number may in part explain differences in the two species' outcrossing rates. Comparable outcrossing rate estimates are available for the narrow endemic *Delphinium viridescens* (Richter, 1993). Bumble bee-pollinated *D. viridescens* is intermediate in size between *D. nuttallianum* and *D. barbeyi* with an average of 46 flowers on a single stalk (Varney, 1979). Outcrossing rates estimated from progeny arrays in five populations of *D. viridescens* averaged 0.717 (range 0.641–0.820; Richter, 1993), also intermediate between the two *Delphinium* species examined in this study. Outcrossing rates estimated for the above and a number of other *Delphinium* species in North America ($N = 1$; Dodd, 1997) and the Mediterranean ($N = 8$; Bosch, 1999) vary widely (0.62–1.0) and do not show an association with floral display size. However, most of these mating system estimates are equilibrium expectations derived from adult fixation indices ($t = (1 - F)/(1 + F)$; Haldane, 1924) and may not reflect the true mating system (see below).

Postpollination events may further act to modify the mating system. Self-incompatibility, autogamy, and inbreeding depression may all influence the success of particular matings, and hence the genetic estimates of outcrossing made at the seed and other stages of the life cycle. *Delphinium barbeyi* produced more seed by autogamy (9%) than did *D. nuttallianum* (2%; Waser, 1978). *Delphinium barbeyi* also appears to be self-compatible, in contrast to its congener, which sets ~40% fewer seed in hand-self vs. hand-outcross pollinations (Waser and Price, 1991). For these reasons differences in the mating system between these two species associated with differences in floral display size will be magnified by differences in their breeding systems.

If inbreeding from geitonogamous pollination has a detrimental effect on fitness, then a greater degree of self-incompatibility may evolve in species with larger floral displays (McDade, 1985). This is clearly not the case with *D. barbeyi* and *D. nuttallianum*, which show the opposite pattern. Likewise, there is no clear relationship between floral display size and the degree of self-compatibility among these and several other *Delphinium* species reported in the literature ($N = 10$). There is a similar lack of association between autogamy rates and floral display size ($N = 19$ species). These aspects of the breeding system appear to be relatively independent of floral display, although annuals have somewhat higher autogamy rates and are more self-compatible than perennials (Macior, 1975; Varney, 1979; Powell and Jones, 1983; Bosch, 1999).

Population genetic structure—We predicted that more intraplant pollen transfer in *D. barbeyi* would result in reduced gene flow and greater genetic differentiation among population subdivisions compared to *D. nuttallianum*. This prediction was strongly supported by the >10 times higher estimates of θ at all spatial scales in the hierarchical F statistics analysis and more pronounced local genetic structure indicated by spatial autocorrelation for *D. barbeyi*. Reviews of genetic structure studies based on allozyme variation indicate that the mating system is one of the strongest determinants of genetic differentiation across a wide range of plant taxa (Heywood, 1991; Hamrick and Godt, 1996). Likewise, almost all previous interspecific comparisons of congeneric species and intraspecific comparisons of populations differing in their breeding systems have found greater genetic differentiation among populations of the more inbred taxa (Appendix). The magnitude of genetic differentiation ($\hat{\theta}$) observed in these two sympatric *Delphinium* species is overall lower, and differences between species somewhat less, than seen in the species and populations compared in the Appendix. This may be due in part to the fact that *Delphinium* populations were sympatric and sampled over a much more limited geographic area than previous studies. It may also reflect that differences in geitonogamy and the breeding system between these two *Delphinium* species reduce gene flow less strongly than the somewhat different factors affecting the mating system and genetic structure in the other species.

Two comparable studies, one from 24 populations of *Delphinium variegatum* from San Clemente Island (Dodd, 1997) and the other from 17 populations of the narrow endemic *D. viridescens* (Richter, 1993; Richter, Soltis, and Soltis, 1994),

TABLE 8. Averages of single allele autocorrelation coefficients, Moran's I , in populations of *Delphinium barbeyi* and *D. nuttallianum*. Data for *D. nuttallianum* are from Williams and Waser (1999). The number of plants, number of loci, and the total number of alleles used to calculate mean autocorrelation coefficients in each population are shown. The coefficient of the least common allele at each locus was not included in the calculation of the mean. Average coefficients are presented for nearest neighbors (I_{NN}), and in 1-m distance intervals. The number of single-allele coefficients used in calculating means that were significantly different from random expectations at $P < 0.05$ (two-tailed test) are indicated as superscripts following each average. Negative superscripts indicate significant negative coefficients.

Population	N loci	N alleles	N plants	I_{NN}	Average Moran's I in each distance class									
					0-1 m	1-2 m	2-3 m	3-4 m	4-5 m	5-6 m	6-7 m	7-8 m	8-9 m	9-10 m
<i>D. barbeyi</i>														
KPA	3	7	75	0.24 ⁴	0.28 ³	0.02 ⁰	0.03 ¹	-0.08 ⁻¹	-0.06 ⁰	-0.05 ⁻¹	0.00 ¹	-0.02 ⁻¹	-0.03 ⁻¹	-0.04 ⁰
KPB	3	7	124	0.16 ⁴	0.09 ²	0.07 ³	-0.02 ⁻¹	0.01 ⁰	-0.02 ⁰	-0.03 ¹⁻²	0.00 ⁰	-0.04 ⁰	-0.00 ¹	-0.02 ⁻¹
KPC	3	7	87	0.15 ³	0.14 ²	0.00 ⁰	-0.03 ¹⁻²	0.01 ¹	0.05 ¹	0.03 ²	-0.05 ⁻¹	-0.07 ⁻¹	-0.03 ⁰	-0.03 ¹⁻¹
BPB	3	7	81	0.28 ⁶	0.35 ⁴	0.13 ²	-0.01 ⁰	-0.08 ⁻¹	-0.02 ⁰	-0.01 ⁻¹	-0.09 ⁻¹	-0.02 ⁻¹	-0.07 ⁰	0.01 ⁰
CCC	3	6	81	0.08 ⁰	0.10 ²	0.05 ²	0.01 ²	-0.04 ⁻¹	-0.01 ⁰	0.04 ²	0.04 ¹	0.00 ⁰	-0.07 ⁻²	-0.02 ⁻¹
<i>D. nuttallianum</i>														
JFA	4	8	324	-0.01 ⁰	0.02 ⁰	-0.01 ⁰	0.02 ¹	0.00 ⁰	-0.01 ⁰	0.00 ⁰	-0.01 ⁰	-0.01 ⁻¹	0.01 ⁰	0.00 ⁰
JFB	4	7	340	0.00 ⁰	-0.01 ⁰	0.00 ⁰	0.00 ⁰	-0.00 ⁰	-0.01 ⁰	0.01 ¹	-0.01 ⁰	-0.00 ⁰	-0.01 ⁰	0.00 ⁰
KPA	4	7	297	-0.01 ¹	0.02 ¹	0.00 ¹	0.02 ¹	0.02 ¹	0.00 ¹	0.02 ³	0.01 ¹	-0.01 ⁰	-0.02 ⁻²	-0.00 ⁻¹
KPB	4	7	339	0.16 ⁴	0.09 ⁶	0.02 ¹⁻¹	0.01 ²⁻²	-0.01 ²⁻²	0.03 ⁴	0.01 ⁰	0.01 ²	-0.02 ⁻¹	-0.00 ¹	-0.01 ⁻²

further support the relationship between floral display size and spatial genetic structure. *Delphinium variegatum* is of similar size to *D. nuttallianum* and has a similar low estimate of F_{IS} (0.067) and a somewhat higher F_{ST} estimate (0.047). The geographic range of sampling for this species was ~25 km. *Delphinium viridescens* has a single inflorescence and is intermediate in total floral display size (46 flowers) between *D. nuttallianum* and *D. barbeyi*. It has somewhat higher genetic differentiation among populations ($\hat{F}_{ST} = 0.209$) than does *D. barbeyi* ($\hat{\theta}_{SITE} = 0.126$), although populations were more widely dispersed (1-30 km) than in our study (1-3 km). For these four genetically well-characterized *Delphinium* species the predicted relationships among floral display size, mating systems, and population genetic structure appear to hold. However, there is no significant association between floral display and genetic differentiation among populations when five additional *Delphinium* species studied by Bosch (1999) are considered.

A puzzling result was the high heterozygosity observed in adult *D. barbeyi* despite low outcrossing rates estimated from seeds. This result also appears at odds with other intrageneric and intraspecific studies in which the more inbred taxa have higher estimates of F_{IS} (Appendix). This suggests that significant changes in the genetic composition of the populations occur between the seed and adult stages of the life cycle of *D. barbeyi*, but not *D. nuttallianum*. An increase in heterozygote frequency throughout the life cycle has been found in numerous studies of genetic demography (e.g., Kahler, Clegg, and Allard, 1975; Tonsor et al., 1993; Hossaert-McKey et al., 1996) and suggests selection for heterozygosity. Schoen (1982), Glover and Barrett (1987), and Holtsford and Ellstrand (1989) also found a pattern of excess heterozygosity in adults relative to inbreeding equilibrium expectations in selfing populations, while outcrossing populations showed slight heterozygote deficits. A similar pattern is seen in the comparison of highly outcrossing *Plantago lanceolata* and mixed-mating *P. coronopus* (Wolff, 1991; Appendix).

Selection for particular heterozygous genotypes in *D. barbeyi* appears unlikely as the cause of its adult heterozygote excesses because similar patterns are found at all three polymorphic loci studied. Also, population subdivisions differed sufficiently in their genotype and allele frequencies at various spatial scales to produce significant genetic differentiation among them. Gitzendanner and Soltis (2000) point out that genetic variability measures are often correlated among rare and widespread species within a genus. If levels of heterozygosity were conserved among *Delphinium* species, then deviations from outcrossing equilibrium estimates such as seen in these two species might result. However, the excess heterozygosity seen in *D. barbeyi* appears to reflect high overall levels of adult heterozygosity ($H_O = 0.54$; this study) compared to those reported for other 15 *Delphinium* species ($H_O = 0.17$, range = 0.07-0.30; Richter, 1993; Dodd, 1997; Bosch, 1999), including *D. nuttallianum* ($H_O = 0.16$; this study).

Greater demographic change in heterozygosity in *D. barbeyi* relative to *D. nuttallianum* may reflect differences in the magnitude and timing of inbreeding depression in the two species (Husband and Schemske, 1996). Partial self-incompatibility, either from inbreeding depression or maternal control (Waser et al., 1987), strongly reduces seed set in *D. nuttallianum* but not *D. barbeyi*. Early-acting inbreeding depression (on seed set) is expected to be more prevalent in outcrossing popula-

tions, while selfing populations, which may already be purged of some of their genetic load, are predicted to exhibit greater late-acting inbreeding depression (on survival and reproduction) (Husband and Schemske, 1996). Because the mating system is estimated from surviving seed, early selection against selfing may reduce the differences in fixation indices estimated from seed and adults in *D. nuttallianum* more than *D. barbeyi*. Alternatively, changes in the mating system over generations or year-to-year variation in the outcrossing rate could explain the discrepancy between selfing rate and adult heterozygosity in *D. barbeyi*.

Conclusions—This interspecific comparison of two *Delphinium* species has demonstrated that floral display is associated with predicted changes in pollinator foraging behavior, the mating system, and population genetic structure. Comparable studies of two other North American *Delphinium* further suggest that species with larger floral displays are more self-pollinated and have reduced gene flow. However, these results should be interpreted with caution. Differences in the breeding systems (autogamy and self-compatibility) of these species may exaggerate the influence of floral display on the estimated genetic parameters. A brief survey of *Delphinium* species suggest that breeding systems vary widely and are not strongly associated with floral display size. More detailed intraspecific studies of variation in plant size and its effects on pollinator behavior and outcrossing rates are needed to understand the trade-offs in the evolution of floral display size. The wide variation in floral display size and long-lived perennial habit of *Delphinium barbeyi* provides an excellent system in which to test these relationships. Likewise, further interspecific studies of floral display size, breeding systems, outcrossing rates, and spatial genetic structure are necessary to gain a better understanding of their relationships. The genus *Delphinium* provides ample opportunities for such comparisons. Over 60 species of *Delphinium* occur in North America, of which *D. nuttallianum* and *D. barbeyi* represent the extremes of floral display size. These two species are placed in different subsections in the most recent taxonomic treatment of North American *Delphinium* (Warnock, 1997), which may introduce historical factors as a potential cause of their breeding system and genetic differences. Future studies of the relationships between floral display, the breeding system, outcrossing rates, and genetic structure should capitalize on the wide range of variation in *Delphinium*, especially if combined with phylogenetic information allowing sister taxa to be compared.

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APPENDIX. Inter- and intraspecific comparisons of fixation within populations (F_{IS}) and genetic differentiation among population subdivisions (F_{ST} or G_{ST}) among taxa differing in their breeding systems or outcrossing rates (t). Standard errors of estimates, when available, are shown in parentheses. When separate estimates from different populations are reported, the means are shown with their ranges in square brackets.

Taxa	No. pop.	No. loci	t	F_{IS}	F_{ST} or G_{ST}	Reference
A) Interspecific comparisons						
<i>Phlox drummondii</i>	73	5	~1.0 ^b	0.408 ^c	0.208 ^c	Levin, 1978
<i>Phlox roemariana</i>	15	4	~1.0 ^b	0.423 ^c	0.213 ^c	
<i>Phlox cuspidata</i>	43	5	0.22 ^{b,d}	0.680 ^c	0.400 ^c	
<i>Oenothera grandis</i>	26	6	~1.0 ^b	0.04 ^c	0.08 ^c	Ellstrand and Levin, 1980
<i>Oenothera mexicana</i>	11	6	≪1.0 ^b	1.0 ^c	0.00 ^c	
<i>Plectritis congesta</i>	15	8	0.702 ^f (0.048)	0.19 [-0.04-0.41]	0.150 (0.039)	Layton and Ganders, 1984
<i>Plectritis brachystemon</i>	10	8	0.024 ^f (0.008)	0.96 [0.86-1.00]	0.639 (0.053)	
<i>Plantago lanceolata</i>	7	11	~1.0 ^b	—	0.037	Van Dijk, Wolff, and De Vries, 1988
<i>Plantago coronopus</i>	4	8	1.01 [0.98-1.08]	—	0.070	
<i>Plantago major</i>	4	7	0.22 [0.06-0.60]	—	0.216	
<i>Plantago lanceolata</i>	5	14	~1.0 ^b	0.110 [0.08-0.13]	0.045	Wolff, 1991
<i>Plantago coronopus</i>	4	13	0.75 [0.35-0.99]	0.010 [-0.18-0.15]	0.120	
<i>Plantago major</i>	4	7	0.03 [0.00-0.08]	0.834 [0.75-0.95]	0.277	
<i>Mimulus guttatus</i>	8	12	0.48	—	0.262	Fenster and Ritland, 1992
<i>Mimulus micranthus</i>	5	7	0.16	—	0.343	
<i>Lolium</i> (3 spp.)			~1.0 ^b	0.04	0.099	Loos, 1993
<i>Lolium remotum</i>			≪1.0 ^b	1.0	0.750	
<i>Cyclamen hederifolium</i>	7	6	0.60 [0.30-1.00] ^f	0.329 (0.078)	0.131 (0.013)	Affre and Thompson, 1997
<i>Cyclamen repandum</i>	8	5	0.26 [0.04-0.86] ^f	0.658 (0.153)	0.415 (0.052)	
<i>Leavenworthia stylosa</i>	3	5	~1.0 ^b	0.474 [0.33-0.65]	0.268 (0.087)	Charlesworth and Yang, 1998
<i>Leavenworthia crassa</i>	5	4	≪1.0 ^{b,g}	0.656 [0.53-0.79]	0.213 (0.047)	
B) Intraspecific comparisons						
<i>Gilia achillifolia</i>	4	13	0.79 [0.64-0.96]	0.13 [0.04-0.22]	0.17	Schoen, 1982
	3	10	0.29 [0.15-0.42]	0.21 [0.05-0.47]	0.39	
<i>Eichornia paniculata</i>	6-10	1-6	0.745 [0.47-0.96]	0.088 (0.005)	0.324 (0.010)	Glover and Barrett, 1987
	3	2-3	0.480 [0.29-0.68]	0.275 (0.041)	0.633 (0.009)	
<i>Clarkia tembloriensis</i>	4	3-6	0.71 [0.51-0.87] ⁱ	0.27 [0.07-0.65] ⁱ	0.42	Holtsford and Ellstrand, 1989
	3	2-3	0.11 [0.03-0.26]	0.82 [0.61-1.0] ⁱ	0.71	
<i>Campanula punctata</i>	7	17	0.743 [0.64-0.79] ^{a,f}	0.163 [0.13-0.23]	0.140	Inoue and Kawahara, 1990
	10	17	0.342 [0.16-0.53] ^{b,d,j}	0.534 [0.33-0.73] ^j	0.308	

^a Self-incompatible = obligate outbreeder.

^b Self-compatible = selfed or mixed-mating system.

^c Calculated as mean of single-locus estimates.

^d Outcrossing rate estimate from one population.

^e Monomorphic at all loci studied.

^f Calculated from $t = (1 - F)/(1 + F)$ averaged over populations.

^g Breeding system estimated from degree of self-compatibility and autogamy rate.

^h Outcrossing (O) vs. selfing (S) populations.

ⁱ Means of maternal fixation indices within populations.

^j Estimates from two self-incompatible island populations were omitted.