THE EFFECT OF PARTIAL SELF-INCOMPATIBILITY ON THE BREEDING SYSTEM OF CAMPANULA RAPUNCULOIDES L. (CAMPANULACEAE) UNDER CONDITIONS OF NATURAL POLLINATION

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The consequences of partial self-incompatibility on the breeding system of Campanula rapunculoides is considered under conditions of natural pollination. We propagated two partially self-incompatible genotypes of C. rapunculoides and established them in pollinator exclusion cages along with 18 donor plants that differed from the focal plants (recipients) in their genotype at the glucose-1-phosphate isomerase (GPI) locus. Cages of plants were opened for pollinator access for 1 h/d, 24 h every fourth day, or left continuously open (control treatment), and the experiment was carried out in two consecutive years (1997 and 1998). After flowering, recipient plants were scored for percentage fruit set and mean seed number per fruit, and then seeds were screened for paternity (self or outcross). ANOVA analyses showed that the mean number of seeds per flower was significantly greater in 1998 than in 1997 and that it was affected by the amount of pollinator access. ANOVA analyses further show that selfing rates were higher in the treatment in which pollinator access was open 24 h every fourth day, while the open-1-h/d treatment did not differ from control cages in any aspect of the breeding system. Selfing rates were higher overall in 1998 than in 1997. We also find a weak effect of the genotype of the recipient and the position of the flower on the selfing rate, although the first result is not unexpected because the genotypes used in the experiment showed similar levels of self-fertility. Our data show that both between- and within-years selfing rates can respond to environmental variation in pollinator availability in C. rapunculoides.

Keywords: breeding system, Campanula rapunculoides, partial self-incompatibility, self-incompatibility, selfing rate.

Introduction

The evolution of breeding systems in plants is often viewed as a balance between the adverse consequences of selfing (inbreeding depression and the loss of opportunities to pollinate conspecifics) and the benefits of selfing (a genetic transmission advantage and reproductive assurance when the availability of cross pollen limits seed production) (Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Lloyd 1992; Uyenoyama et al. 1993; Barrett and Harder 1996). In many species of flowering plants, selfing is often prevented by a genetic self-incompatibility (SI) system under the control of the gene products of the S-locus, whereby self-pollen is recognized and rejected by the pistil before fertilization (de Nettancourt 1977). Over the past 2 decades, there have been tremendous advances in our understanding of the molecular, biochemical, and cellular processes underlying SI (Dzelzkalns et al. 1992; Clarke and Newbigin 1993; Sims 1993; Franklin et al. 1995; Newbigin 1996; Hiscock and Kies 1999; McCubbin and Kao 1999; Dickinson 2000). We now know that the major forms (including heteromorphic and gametophytic and sporophytic homomorphic) of SI are independently derived and there appears to be multiple origins within each of the major morphic forms (Barrett 1992; Franklin et al. 1995; Kohn et al. 1996; Hiscock and Kies 1999).

Although some SI species show strict SI, other species or populations of plants with functional SI systems show plasticity or variation in the strength of SI. Genetic variation in the expression of SI can be caused by the action of specific S-alleles (weak, strong, and null alleles) or the expression of unlinked modifier genes (Levin 1996). Moreover, the expression of SI in many species is also influenced by external environmental conditions, such as temperature, and internal stylar conditions, such as the age of the flower or presence of developing fruits (Ascher 1976; Bowman 1987; Anderson et al. 1989; Holland and McNeilly 1990; Richardson et al. 1990; Becerra and Lloyd 1992; Kron et al. 1993; Jakobsen and Martens 1994; Jones 1994; Levin 1996; Vogler et al. 1998). These variations in the expression of SI probably represent a constellation of related phenomena involving subtle pollen-pistil interactions that affect the relative performance of self and cross pollen. Recently, a few evolutionary theorists have begun to view variation in the expression of SI as a component in the origin and maintenance of mixed mating systems (Levin 1996; Stephenson et al. 2000). However, little is known about the consequences of variation in the strength of SI on the breeding systems of the plants possessing them.

Previous studies of plants from two natural populations of
**Campanula rapunculoides** have shown that the flowers of most individuals are highly self-incompatible when the stigmas first become receptive, but many become more self-fertile as the flowers age (Richardson et al. 1990; Vogler et al. 1998) and that there is genetically based variation in the strength of SI in young and old flowers (Good 2000). Specifically, these studies have shown that (a) self-pollination of young flowers (first day of stigma receptivity) usually results in very low fruit and seed set; (b) self-pollination of old flowers (fourth day of stigma receptivity) usually results in an increase in fruit and seed set; (c) cross-pollen tubes grow significantly more rapidly than self tubes in the styles of young flowers, but the differences in growth of cross- and self-pollen tubes decreases significantly in the styles of old flowers; (d) differences in pollen-tube growth rates lead to differences in the probability of fertilization when equal mixtures of cross pollen and self pollen are deposited onto stigmas; and (e) there is significant broad and narrow sense heritability for the strength of SI and its breakdown with floral age (Richardson et al. 1990; Stephenson et al. 1992, 2000; Vogler et al. 1998).

In this study, we investigate the consequences of variability in the strength of SI (partial self-incompatibility) on the breeding system of *C. rapunculoides* under conditions of natural pollination. Specifically, we experimentally varied pollinator availability (access of pollinators to the plants) in artificial populations bearing a genetic marker. We hypothesized that outcrossing should predominate when pollinator activity is great and self-pollination should increase when there is little pollinator activity. Because within- and between-year variations in pollinator activity are common features in natural populations (Stephenson et al. 1995), this study examines the effects of an environmentally relevant variable on the breeding system, and it differs from our previous studies in that it relies on the foraging of natural pollinators and on natural patterns of pollen deposition (e.g., mixed and pure pollen loads, variations in the ages of flowers at the time of pollen deposition, etc.).

**Material and Methods**

**Experimental Species**

*Campanula rapunculoides* L. (Campanulaceae) is a naturalized perennial herb, locally abundant along roadsides and open woods across the northeastern United States and Canada (Rosatti 1986). It overwinters as a rosette, and in July, each rhizomatous cluster produces one to eight bolts (flowering racemes) containing 20–70 blue, bell-shaped flowers that open acropetally (bottom upward). *Campanula* is dichogamous: when the flower first opens, pollen is deposited onto specialized stylar hairs (Nyman 1993) and the stigmatic lobes remain tightly appressed. Thus, at anthesis, the flower is phenotypically male and cannot be pollinated. After a few days, during which the pollen is removed by bees (primarily *Bombus* and *Apis*), the stigmatic lobes reflex and the flower becomes phenotypically female. Richardson and Stephenson (1989) demonstrated that pollen removal shortens the duration of the male phase. Both geitonogamous and autogamous self-pollination can occur. Several male- and female-phase flowers are usually open simultaneously on each inflorescence, so self pollen can be transported within an inflorescence. In addition, because a plant may produce more than one bolt, geitonogamy can occur between inflorescences. The female phase lasts 4–5 d and is characterized by the progressive curling of the stigmatic lobes until they come into contact with the style on the fourth or fifth day after stigmatic opening. Therefore, delayed autogamous self-pollination is also possible (if there is still pollen on the style).

**Experimental Design**

The nuclear copy of the GPI (glucose-1-phosphate isomerase) locus from plants collected from two natural populations of *C. rapunculoides* (near Bellefonte, Pa., and near Duansburg, N.Y.) was examined using cellulose acetate gel electrophoresis (CAGE). From this screen of individuals at the GPI locus, we identified 18 “donor” plants that were completely cross-compatible with, and bore alternative GPI alleles to, two “recipient” plants. By dividing the rhizomatous rootstock of these 20 plants, growing them in 4-L pots in a greenhouse, and then redividing the rootstock, we were able to make multiple clones of each of the 20 plants (18 donors and two recipients). The clones were then placed into a cold room for 60 d (in order to synchronize their growth/reproductive cycles) before being returned to the greenhouse. When the plants began to bolt, they were transferred to a field site (Pennsylvania State University Agriculture Experiment Station, Rocksprings, Pa.) and placed into six 2 × 2 × 2-m cages that were covered with 30% shade cloth to provide appropriate shade for the plants while effectively preventing access by the pollinators. No cage was placed within 100 m of another cage and two cages were randomly assigned to each of three “pollinator access” treatments: pollinator access for 1 h/d (1-h treatment), 24 h every fourth day (every-fourth-day treatment), and open pollination (control treatment). These treatments were considered to roughly emulate conditions of low (1 h/d) and sporadic (1 d of every 4 d) pollinator activity. The three treatments were created by rolling up the sides (shade cloth) of the cages for the appropriate time each day. Therefore, both treatment and recipient within treatment were replicated, and the entire experiment was conducted in two consecutive years (1997 and 1998). In 1997, the inflorescence of one clone of recipient 2 in a control cage was destroyed leaving only one clone of recipient 2 for the control treatment in this year.

After flowering, the recipient plants were transported to a greenhouse where the fruits continued to develop. At maturity, percentage fruit maturation was calculated (number of mature fruits/number of flowers), the fruits were harvested, and the seeds were counted. The infriuctescence of each recipient clone was divided into thirds (bottom, middle, and top), and random samples of 33 (1997) or 36 (1998) seeds from each third (giving a total of 1089 seeds in 1997 and 1296 in 1998) were screened for paternity (self or outcross) based on their alleles at the GPI locus using CAGE. Seeds were ground in extraction buffer (5 mL 0.09 M Tris, pH 8.0, 5 mL ddh20, 0.2 g PVP-40, 15 mg DTT), subjected to electrophoresis through cellulose acetate gels (Helena Laboratories) for 15 min at 200 V, and stained for GPI using the protocol provided by the gel manufacturer. Seeds were considered to be aborted if they did not give an isozyme band and appeared effete. The recipient plants...
(two genotypes) were selected because they were homozygous for a fast nuclear allele at GPI, while the donor plants were polymorphic at other alleles such that an outcrossing event is definitely determined by the presence of more than one band in the progeny. We examined whether there was a correlation between the number of aborted seeds and (a) the proportion of selfed or outcrossed seeds and (b) the experimental treatment and found none. Consequently, we ignored the aborted seeds and calculated the selfing rate, s, as the number of selfed seeds/(number of selfed + outcrossed seeds) in the sample.

Finally, one clone of each donor plant and several clones of each recipient genotype were left in the greenhouse, and every other flower on each recipient plant was outcrossed to each donor plant. The age of the flowers for these outcross pollinations varied between young (day 1 of the female phase) and old (day 4 of the female phase). These pollinations were repeated until the supply of flowers was exhausted. In addition to the outcrosses, nine flowers on recipient 1 (10 on recipient 2) were selfed in both the young and old female phases. These pollinations allowed us to determine seed number per outcrossed/selfed fruit on each recipient and to check that each donor sired offspring on the recipient plants that were clearly identifiable from selfed progeny at the GPI locus.

Statistical Analyses

Least squares means of seed and fruit set were calculated for greenhouse- and field-pollinated fruits. The difference between the percentage seed set after hand self-pollination on young and old female flowers, the difference in seed set after hand cross-pollination and open pollination between the percentage seed set after hand self-pollination on young and old female flowers, the difference in seed set after open pollination and greenhouse-pollinated fruits. The difference between the percentage seed set after hand self-pollination on young and old female flowers, the difference in seed set after hand cross-pollination and open pollination were assessed with standard t-tests. To examine whether there was a significant influence of year, treatment, or genotype on seed set, we ran a fixed-effect ANOVA on the mean number of seeds per flower with main effects year, treatment, and genotype (recipient 1 or 2). To examine the factors influencing the selfing rate, we ran another fixed-effect ANOVA on the mean selfing rate, s, with main effects year, treatment, genotype, and position (bottom, middle, or top of the infructescence) with the interaction terms treatment × year, position × year, genotype × position × year, and treatment × position × year. All other interactions contributed little to the sum of squares and were included in the error term. The mean selfing rate, s, was square root transformed before analyses to meet the assumption of normality for the latter ANOVA analyses. Plots of the mean selfing rate and their standard error for each of the four main effects are presented. Hypotheses concerning differences between the mean number of seeds per flower or the realized selfing rate among treatments were analyzed using Type III sums of squares as defined by the program MiniTab. To assess the direction of effects deemed significant by the ANOVA analyses, pairwise comparisons using Tukey corrections with an overall α of 0.05 were used.

Results

Seed and Fruit Set

Under greenhouse conditions, both recipient genotypes produced significantly more seeds when outcrossed than when self-pollinated. On genotype 1, self seed set did not significantly decline over floral age and was 59.2 ± 7.3 on young flowers and 66.7 ± 15.8 on older flowers. On genotype 2, self seed set was 47.4 ± 9.1, following self-pollinations on young flowers and 79.2 ± 13.4 on older flowers (t = 2.49, df = 19, P < 0.05). Genotype 2 produced significantly more outcrossed seeds per fruit than did genotype 1 after hand cross-pollination (mean = 108.3 ± 4.4, mean = 119.7 ± 5.4; t = 4.18, df = 98, P < 0.0001). Both genotypes produced more seeds per fruit after hand cross-pollination in the greenhouse than after open pollination (control cages) in the field (mean = 61.3 ± 2.7, mean = 72.8 ± 2.1; t = 3.22, df = 69, P < 0.0001, genotype 1; t = 6.67, df = 73 P < 0.0001, genotype 2).

Mean seed set per fruit was 37.66 ± 8.5 (SE) in 1997 and 68.86 ± 5.6 in 1998, while mean fruit set was 67.1 ± 7.8 in 1997 and 91.9 ± 3.4 in 1998. To examine the variation due to both factors, we performed ANOVA analyses on the mean number of seeds per flower (table 1). The ANOVA results in table 2 show that mean seed number per flower varied significantly between years. Tukey pairwise comparisons indicate that there were significantly more seeds per flower in 1997 than in 1998 (mean = 27.33 ± 4.1, mean = 64.75 ± 4.1; t = 5.36, P < 0.0001; see also table 2). An ANOVA also shows that there was a significant effect of the treatment on the mean number of seeds per flower (table 2). Tukey pairwise comparisons indicate that recipients in the every-fourth-day treatments produced fewer seeds per flower than control cages (mean = 38.9 ± 5.0, mean = 55.6 ± 5.6; t =

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Recipient 1</th>
<th>Recipient 2</th>
<th>Overall</th>
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<tr>
<td>1997</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Open pollination</td>
<td>104 39.2 ± 3.4</td>
<td>73 51.6 ± 2.4</td>
<td>104 39.2 ± 3.4</td>
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<tr>
<td>H/d</td>
<td>81 16.7 ± 3.8</td>
<td>73 26.1 ± 3.1</td>
<td>81 16.7 ± 3.8</td>
</tr>
<tr>
<td>Every fourth day</td>
<td>84 11.3 ± 2.1</td>
<td>102 23.3 ± 2.7</td>
<td>84 11.3 ± 2.1</td>
</tr>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open pollination</td>
<td>92 63.1 ± 4.1</td>
<td>105 74.4 ± 4.9</td>
<td>92 63.1 ± 4.1</td>
</tr>
<tr>
<td>H/d</td>
<td>101 52.6 ± 2.1</td>
<td>71 79.7 ± 6.2</td>
<td>101 52.6 ± 2.1</td>
</tr>
<tr>
<td>Every fourth day</td>
<td>89 69.20 ± 4.7</td>
<td>69 49.6 ± 5.5</td>
<td>89 69.20 ± 4.7</td>
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2.94, \( P = 0.009 \)), while there was no difference between the mean number of seeds per flower in the 1-h/d and control cages (mean\textsubscript{1h} = 44.09 ± 5.4; \( t = 0.81, P = 0.43 \)).

**Selfing Rate**

The ANOVA on the selfing rates, \( s \), shows that treatment (pollinator access) has a significant effect on the selfing rate (table 3). Even though there are marginally nonsignificant treatment \( \times \) year and position \( \times \) year interactions (table 3), it is reasonable to conclude that the treatment effect alone significantly influenced selfing rates because within-year ANOVAs (not presented) also show that treatment is significant. Pairwise comparisons with Tukey corrections for multiple comparisons indicate that the recipients in the every-fourth-day treatment had significantly higher selfing rates than in the control cages but recipients in the open-1-h/d treatment did not (mean\textsubscript{fourth-day} = 0.64 ± 0.03; mean\textsubscript{control} = 0.30 ± 0.02; \( t = 5.7, P < 0.0001 \); mean\textsubscript{1h} = 0.38 ± 0.03 not different from control \( t = 1.5, P = 0.14 \)). But, in the ANOVAs performed separately on years (not shown), the selfing rate in the every-fourth-day treatment was significantly higher than the 1-h/d in 1997 but not in 1998 (1997 mean\textsubscript{1h} = 0.27 ± 0.05, mean\textsubscript{fourth-day} = 0.64 ± 0.04; \( t = 4.6, P < 0.0001 \); 1998: mean\textsubscript{1h} = 0.49 ± 0.04, mean\textsubscript{fourth-day} = 0.65 ± 0.04; \( t = 2.25, P = 0.10 \)) (see fig. 1A, 1B). The selfing rates in the open-1-h/d and control cages did not differ in any analysis. The selfing rate also differed by year. Selfing rates were higher in 1998 than in 1997 (mean\textsubscript{1997} = 0.37 ± 0.03, mean\textsubscript{1998} = 0.50 ± 0.02; \( t = 2.82, P = 0.006 \)) (fig. 1C). There was no overall significant effect of floral position on the selfing rate, but there was a tendency for selfing rates to decline as the position of the flower increased with respect to the inflorescence (fig. 1D). There was no significant difference in the selfing rate between the two recipient genotypes.

Finally, Spearman rank correlations show that there is no correlation between the selfing rate and the mean number of seeds per fruit \( (r^2 = 0.00, P = 1.0) \) or with the proportion of flowers that set fruit \( (r^2 = 4.4%, P = 0.18) \), although there was a tendency for plants with higher selfing rates to have lower fruit set. Fruit set, however, was highly correlated with seed number per fruit \( (r^2 = 25.8%, P < 0.009) \).

**Discussion**

This study examines the effects of variable pollinator access on the breeding system of the partially self-incompatible species, *Campanula rapunculoides*. We found that, in two separate years, the proportion of selfed seeds varied with pollinator access (open-pollination cages < 1-h/d cages ≤ every-fourth-day cages). Moreover, the selfing rate varied by year, and it tended to vary by floral position within a given year (selfing rates tended to decline with increasing floral position) (fig. 1D). In the control cages in 1997, only 25% of the seeds were the result of self-fertilization, while in the open every-fourth-day cages, two-thirds of the seeds were the result of self-pollination in both years. In short, this study found a >40% difference in the selfing rate of plants growing in very similar populations (similar genetic composition, floral densities, plant densities, and pollinator faunas) when pollinator availability was experimentally varied.

Over the past 2 decades, many studies using a variety of species have found that pollinator availability in natural populations of plants can vary within years, between years, and among populations (Horovitz and Schemske 1988; Willson and Thomson 1991; Stephenson et al. 1995; Ramsey and Vaughn 1996; Stout et al. 1998). Other studies have found that selfing rates in natural populations can also vary within years, between years, and among populations (Belauussoff and Shore 1995; Johnston and Schoen 1996). Studies have reported that the mean selfing rate among individuals in the same population can be affected by variation in floral presentation such as the position of a flower (Barrett et al. 1994; Navarro 1997), the number of open flowers (de Jong et al. 1992a; Brunet and Eckert 1998), or the number of simultaneously flowering ra-

<table>
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<th>Source of variation</th>
<th>df</th>
<th>Adjusted MS</th>
<th>F</th>
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<tr>
<td>Year</td>
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<td>22143.0</td>
<td>36.42***</td>
</tr>
<tr>
<td>Treatment</td>
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<td>1638.8</td>
<td>2.70*</td>
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<tr>
<td>Genotype</td>
<td>1</td>
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<td>1.82</td>
</tr>
<tr>
<td>Error</td>
<td>65</td>
<td>608.1</td>
<td></td>
</tr>
</tbody>
</table>

Note. Main effects are treatment, genotype, position, and year. Interactions for four two-way interactions and one three-way interaction potentially relevant to the biological significance of variation in selfing rates were calculated, other interactions are included in the error term. Model \( r^2 = 0.61 \).
rather than to examine within-population dynamics of species with intermediate selfing rates. This tendency is even more pronounced if the species possesses a genetic SI system. In particular, few studies have examined how environmental variation in pollinator availability can influence selfing rates. This is somewhat surprising because variation in pollinator abundance has been considered one of the primary ecological variables that could select for intermediate selfing rates (Lloyd 1980, but see Morgan et al. 1997). Our study clearly demonstrates that the selfing rate in a population of plants that are partially self-incompatible can vary dramatically with pollinator availability.

Although the recipients in the control cages in our study had a lower selfing rate than the experimental cages, we were surprised at the relatively high rate of selfing (25% and 42% in 1997 and 1998, respectively) in the control cages—especially in light of our previous finding that cross pollen is more likely to sire seeds than self pollen when self- and cross-pollen mixtures are deposited onto stigmas (Stephenson et al. 2000). The high rate of selfing is probably due to high self-fertility of recipient genotypes used in this study. Hand self seed set on the two recipients was approximately 55% and 40% of the cross-seed set following pure self and pure cross-pollination on young flowers and 62% and 66% on old flowers in the greenhouse (see table 1). A survey of plants in two natural populations of C. rapunculoides revealed that only a small percentage of plants are more self-fertile than this, while the majority of plants are more strongly self-incompatible (Good 2000). Furthermore, the similarity of the two genotypes with respect to their level of self-fertility explains why we did not detect a significant effect due to the genotype in the ANOVAs. If our recipient plants had represented the full spectrum of self-fertilities in plants sequestered from natural populations, we would probably have seen significant variation in selfing rates due to the genotype of the recipient, as other studies from our laboratory group have found using hand pollinations (Vogler et al. 1998; Good 2000).

Given that the recipients have a self-fertility of 62%–66% in old female phase flowers and that the selfing rate was $s = 0.66$ for both recipients in both years in the open every-fourth-day treatment, it is likely that many of the flowers in this treatment were self-pollinated when they were old. Thus, even though our focal plants were weakly SI, the fact there is a breakdown in SI in most genotypes from natural populations of C. rapunculoides implies that self-fertilization in old female flowers may be an important facet of the mating system in natural populations. Theoretical results show that if ovules remain available (i.e., unfertilized) after opportunities for outcrossing have occurred, genes leading to delayed self-fertilization (genes that promote autogamy late in the life of a flower) will always be selected (Lloyd 1979, 1992) unless the cost of producing selfed progeny decreases survivorship in perennial species (Morgan et al. 1997).

In this study, both recipients produced significantly more seeds upon outcrossing in the greenhouse than upon open pollination in the field. These findings may indicate that seed set

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**Fig. 1** Mean and standard error of the mean selfing rate for three of the main effects: A, treatment (1997); B, treatment (1998); C, year; D, position.
in the cages was limited by the availability of pollen, even in the control cages. It should also be noted that the control and experimental cages consisted of small populations (only 18 donor plants and two recipients) that were isolated from other populations of C. rapunculoides. Many studies have shown that population/floral density influences pollinator attraction, pollinator foraging behavior, and pollen-dispersal patterns (Stephenson 1979; Nakamura et al. 1989; Devlin et al. 1992; Inoue et al. 1995; Andersson 1996; Conner and Rush 1996; Ushimaru and Kikuzawa 1999). If pollen availability did limit seed set in the control cages, then self pollen would (at least sometimes) not be in competition with cross pollen for access to the ovules. In short, the combination of using relatively self-fertile plants as recipients (chosen because they had appropriate/identifiable GPI alleles), small population sizes, and pollen-limited seed production probably prevented us from finding the full range (i.e., $s < 0.25$) of selfing rates that we might expect in a natural populations exposed to various rates of pollinator visitation. Nevertheless, even under the conditions of this study, we found outcrossing rates that varied from predominantly outcrossing ($s = 0.25$) to predominantly selfing ($s = 0.68$) when pollinator access was experimentally varied.

Finally, C. rapunculoides is a perennial herb that inhabits roadsides, open woods, and disturbed habitats (Rosatti 1986). A previous study from our lab has shown that there are high levels of inbreeding depression in C. rapunculoides (Vogler et al. 1999). Although inbreeding depression was found to be highly variable among families (ranging from 0.24 to 0.98) and less severe in the families derived from plants with weak SI (Vogler et al. 1999), it is unlikely that there would be strong selection for self-fertilization in large populations with high rates of pollinator visitation. However, the data from the study reported here indicate that when population size is small and pollinator service is inadequate (as is likely to occur periodically in populations of weedy colonizers), there could be strong selection for self-fertility. Although inbreeding depression undoubtedly influences the evolution of self-fertilization, more studies and models on the role of pollinator fluctuations in the evolution of mixed mating systems are warranted (Lloyd 1992; Sakai 1996).

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**Literature Cited**


Stephenson A 1979 An evolutionary examination of the floral display of Catalpa speciosa (Bignoniaceae). Evolution 33:1200–1209.


