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# EXPLAINING OUTCROSSING RATE IN *CAMPANULASTRUM AMERICANUM* (CAMPANULACEAE): GEITONOGAMY AND CRYPTIC SELF-INCOMPATIBILITY

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A flexible mating system may enable self-compatible plants to prevent self-fertilization under certain circumstances. *Campanulastrum americanum* is a self-compatible, protandrous herb. Although within-plant pollen transfer is likely and self pollen can produce a full set of seeds, selfing in natural populations is rare. We investigated explanations for the high outcrossing rate. One possibility was that self pollen contacts the stigma rarely. We surveyed floral displays in nature to gauge the potential for within-plant pollen transfer. Floral displays in nature are large enough to enable frequent geitonogamous pollinator visits and subsequent self-pollination. Alternatively, the high outcrossing rate could result from cryptic self-incompatibility, a mechanism that favors outcross pollen over self pollen. To determine whether outcross pollen has a seed-siring advantage over self pollen, we pollinated maternal plants with equal mixtures of self and outcross pollen. Genotyping the offspring revealed that outcross pollen sired significantly more seeds than self pollen. We explored whether differential growth rates of self and outcross pollen tubes produce cryptic self-incompatibility. Growth rates did not differ, indicating that cryptic self-incompatibility occurs by some other mechanism. Cryptic self-incompatibility enables *C. americanum* to reduce inbreeding when outcross pollen is present yet ensure reproduction when only self pollen is available.

*Keywords:* cryptic self-incompatibility, pollen competition, outcrossing mechanisms, geitonogamy, pollen tube growth.

## Introduction

Flowers of hermaphroditic plants commonly receive both self and outcross pollen (Darwin 1876, 1877). Offspring resulting from self-fertilization, however, may suffer reduced fitness because of inbreeding depression (Darwin 1876; Lande and Schemske 1985; Uyenoyama 1986; Charlesworth and Charlesworth 1987; Cruzan and Barrett 1993). Therefore, many species have evolved mechanisms to avoid producing selfed offspring. Some species reduce self-pollination through spatial or temporal separation of male and female flowers or floral morphology that blocks self pollen from contacting the stigma (e.g., Lloyd and Webb 1986; Montalvo 1992; Rigney et al. 1993; Jones 1994; Routley and Husband 2003). Other species, termed self-incompatible, prohibit self pollen from entering the ovules or cause ovules to abort self-fertilized seeds (e.g., Lewis 1979; Richards 1986; Casper et al. 1988; Weller and Ornduff 1989; Montalvo 1992; de Nettancourt 2001). Although avoidance of self-pollination and self-incompatibility prevents plants from investing in less fit offspring, these traits may prove disadvantageous when populations experience conditions that periodically reduce availability of outcross pollen or availability of pollinators to facilitate outcrossing (Baker 1966; Bowman 1987; Barrett and Eckert 1992; Lloyd 1992; Cruzan and Barrett 1993; Johnston 1993; Jones 1994; Eckert and Allen 1997). A flexible mating system that prevents self-fertilization unless it would be advantageous has been argued to be the ideal mating system, especially in circumstances where pollinator visitation is unpredictable.

Recent work has suggested that some self-compatible species may be able to distinguish between self and outcross pollen and prevent self-fertilization under certain circumstances. The autotetraploid *Campanulastrum americanum* is a protandrous herb in which both male- and female-phase flowers can be open simultaneously on the same plant. Geitonogamous pollinations, which transfer pollen from male-phase flowers to female-phase flowers on the same plant, create opportunities for self-fertilization (Galloway et al. 2002). *Campanulastrum americanum* is also self-compatible; self-pollination readily produces viable seeds in near equal numbers to outcross pollination (Galloway et al. 2003). Given these features of the species' floral morphology and reproduction, we would expect selfed progeny to be common in nature. However, an analysis of outcrossing rates in a natural population revealed almost no selfing ($t_m = 0.938$; Galloway et al. 2003).

One explanation for the high outcrossing rate is that self pollen contacts the stigma less often than presumed. Galloway et al. (2002) found a strong positive correlation between floral display size and the number of potentially geitonogamous visits. Geitonogamous visits were also more frequent when plants were farther from conspecifics (Galloway et al. 2002). Therefore, if many plants in natural populations are small with only a few flowers open at a time, pollen movement

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**Table 1**

**Potential for Geitonogamous Selfing in a Natural Population of *Campanulastrum americanum***

| Variable | July 24 | July 28 | August 1 | August 5 | August 10 | Average |
|---|---|---|---|---|---|---|
| Number of plants | 51 | 60 | 63 | 63 | 63 | |
| Capable of selfing (%) | 29.41 | 51.43 | 66.67 | 50.79 | 42.86 | 48.11 |
| For selfing-capable plants: | | | | | | |
| Open flowers | 7.27 | 5.92 | 7.36 | 8.31 | 10.59 | 7.89 |
| Median distance (cm) | 15.67 | 14.67 | 15.00 | 13.83 | 14.67 | 14.77 |

Note. Plants capable of selfing had at least one male-phase flower and one female-phase flower open simultaneously. Number of open flowers and median distance from nearest three plants were averaged for all plants surveyed per date.

from male-phase to female-phase flowers may be limited. Likewise, if distance between plants is typically small, pollinators may visit only a few flowers per plant and thus not facilitate self-pollination.

A second explanation for the rarity of inbred individuals is cryptic self-incompatibility. Bateman (1956) coined the term cryptic self-incompatibility to describe the high relative success of outcross pollen when competing with self pollen. Further studies have found cryptic self-incompatibility in a number of species (e.g., Bowman 1987; Hessing 1989; Cruzan and Barrett 1996; Eckert and Allen 1997). Cryptic self-incompatibility commonly results from faster growth of outcross pollen tubes in the style (Weller and Ornduff 1977, 1989; Glover and Barrett 1986; Hessing 1989; Aizen et al. 1990; Cruzan and Barrett 1996; Eckert and Allen 1997). Later arrival of self pollen tubes at the ovules gives outcross pollen a siring advantage when pollen grains outnumber unfertilized ovules (Cruzan and Barrett 1996). Alternatively, it has been suggested that outcross pollen may exhibit a higher rate of germination (Thomson 1989; Weller and Ornduff 1989; Snow and Spira 1993; Eckert and Barrett 1994; Smith-Huerta 1996; Eckert and Allen 1997) or a superior ability to fertilize the ovules (Weller and Ornduff 1989; Snow and Spira 1993; Eckert and Barrett 1994; Eckert and Allen 1997). The ability of a species to recognize and favor outcross pollen over self pollen avoids inbreeding depression when outcross pollen is available yet enables reproductive success through self-fertilization when necessary.

Here we explore three potential explanations for the high outcrossing rate in *C. americanum*. We first evaluate potential for within-plant pollen transfer by surveying floral displays in a natural population. Next, we examine the possibility of cryptic self-incompatibility in *C. americanum* by comparing rates of seed-siring success for self and outcross pollen when equally represented on the stigma. Finally, we investigate a mechanism of cryptic self-incompatibility by comparing growth rates of self and outcross pollen tubes. The three aspects of this study complement each other to form a comprehensive evaluation of the mating system of *C. americanum*.

## Material and Methods

### Study Species

*Campanulastrum americanum* Small ( = *Campanula americana* L.) is a self-compatible monocarpic herb that flowers between mid-July and late August. The protandrous flowers open in the male phase with pollen presented on the outer surface of the style. Plants in large populations may receive up to eight pollinator visits per 15-min period (Galloway et al. 2002), which results in removal of most of the pollen within 2 h of anthesis and leaves the flower functionally neuter (Evanhoe and Galloway 2002). The stigmatic lobes open the following day, initiating the female phase that continues until pollinators saturate the stigma with pollen. Flowers are located in compact inflorescences at reproductive nodes on the main stem and side branches. Typically, a single flower is open at each node, with flowers at one to six (mean ± SE = 3.05 ± 0.44) adjacent nodes open simultaneously. Anthesis progresses distally along branches; therefore, the lower flowers in blooming clusters are in female phase, while the newly opened flowers toward branch tips are in male phase. The number of clusters of flowers increases with branch number and branch length.

### Floral Display Size in a Natural Population

To determine the potential for self-pollination, we surveyed floral display sizes in a natural population (Route 700, Giles County, VA). Surveys were conducted between July 24 and August 10, 2003, and encompassed the peak flowering period. Surveys included all plants within two 20 × 2-m transects for a total of 63 plants. On the first survey date, the distance from each plant to the nearest three flowering plants was measured. On each survey, we counted the number of flowers per plant in each phase: male, female, and neuter.

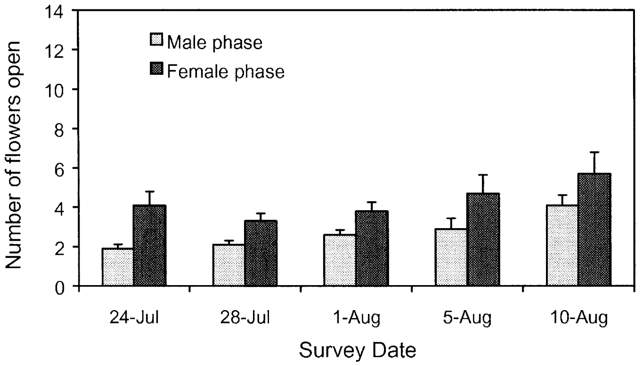

**Fig. 1** Average number and SE of male- and female-phase flowers on wild *Campanulastrum americanum* plants at five dates during the flowering season. Only plants with both male- and female-phase flowers were included.

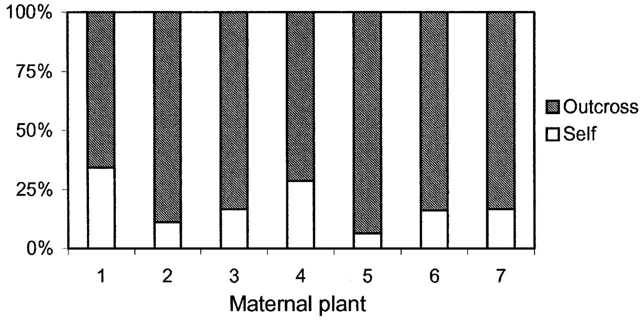

**Fig. 2** Percent of self and outcross progeny of seven *Campanulastrum americanum* plants following pollination with 50% self and 50% outcross pollen.

The population was surveyed every fifth day between 1400 and 1800 hours. Since flowers typically have a life span of 3 d, individual flowers were included in only one survey.

### *Siring Success of Self and Outcross Pollen*

To determine whether cryptic self-incompatibility is present in *C. americanum*, we compared the rates of seed-siring success for self and outcross pollen. Seven maternal plants were selected from the same population used in the floral display surveys on the basis of their genotypes for the allozymes trioephosphate isomerase (TPI-1) or malate dehydrogenase (MDH-2). Twenty-one different plants were selected as pollen donors. Pollen recipients and pollen donors were chosen so that each seed could be identified as the product of a self or outcross mating. *Campanulastrum americanum* is an autotetraploid (Galloway et al. 2003), and relatively few individuals had genotypes that permitted paternity assignment. The morning before pollination, male-phase flowers were emasculated by removing pollen from the outer surface of the style with a wet paintbrush. This ensured that no self pollen contaminated the stigma. When the flowers entered female phase the next day, mixtures of self and outcross pollen were prepared in the indentation of a depression slide. Mixtures consisted of fresh pollen from three male-phase flowers of the maternal plant and one male-phase flower from each of three outcross donors. Outcross donors varied among maternal plants. Pollen mixtures were applied to the stigmas of three to six female-phase flowers per maternal plant. Stigmas were saturated with pollen to ensure pollen competition. Two types of additional pollinations were conducted on each plant, pure self pollen and pure outcross (mixture of the same three donors). Fruits were collected from the pollinated flowers when ripe.

Seeds from pollinations were grown under controlled conditions. Seeds were sprinkled on the surface of 6-cm pots filled with Promix potting medium and germinated in a growth chamber under 12L : 12D. Seedlings were individually transplanted into tubular pots (13.5 cm long, 4 cm diameter) containing three parts Promix and one part Turface and grown in a greenhouse until large enough for genotyping. Siring success was evaluated on seedlings with two or more true leaves at least 1 cm in diameter.

We determined the proportions of selfed and outcrossed offspring produced from mixed pollinations using allozyme electrophoresis. Ca. 100 offspring from each maternal plant were genotyped. A dime-sized leaf from each individual was ground in a spot glass with the extraction buffer of Wendel and Parks (1982). The enzyme was absorbed onto a filter paper wick. Wicks were stored at −80°C until run on a gel. Gels for TPI used Buffer System 8, and gels for MDH used Buffer System 4 (Soltis et al. 1983). TPI gels were run at 200 V for the first half hour, 250 V for the second half hour, and 300 V for the remaining 3.5 h. MDH gels were run at 180 V for 3 h. Gels were stained, incubated for 45 min at 37°C, and scored according to the protocol employed by Galloway et al. (2003). Proportions of selfed and outcrossed offspring were compared with the expected 50 : 50 ratio using a *G*-test of goodness of fit.

### *Pollen Tube Growth Rates*

To determine whether differential pollen tube growth was responsible for the high outcrossing rate, we compared growth rates of self and outcross pollen tubes. Plants came from seeds that were randomly gathered from a population on Route 613 (Giles County, VA) and grown in a greenhouse. Thirty-one maternal plants each received two different pollination treatments: self and outcross. Each pollen type was applied to four flowers per plant. The morning before pollination, male-phase flowers were emasculated to prevent contamination by self pollen. Pollinations took place after the stigmatic lobes had opened, between 1400 and 1700 hours. Flowers were hand-pollinated by swiping a pollen-covered style from a male-phase flower from the same plant (self) or from a random unrelated plant (outcross) across the stigmatic lobes of the female-phase flowers. Since differences in pollen tube growth rates may be more evident in certain growth stages, pairs of self- and outcross-pollinated flowers were collected 2, 3.5, 5, and 24 h after pollination.

Pollen tubes were prepared for viewing using techniques modified from Kearns and Inouye (1993, p. 126). Immediately following collection, the style was separated from the ovary and fixed in 95% ethanol for at least 2 h to halt pollen tube growth. Styles were then placed in 10 M sodium hydroxide for 24 h to soften and clear the tissue. Finally, styles were rinsed in tap water and soaked in 1% aniline blue stain overnight. Because the styles of *C. americanum* are thick, it was necessary to split them longitudinally before mounting

## Table 2

**G-Test Evaluating Equal Siring Ability of Self and Outcross Pollen**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Allozyme system | TPI | TPI | TPI | TPI | TPI | MDH | MDH |
| *N* | 80 | 101 | 18 | 35 | 109 | 81 | 96 |
| *G* statistic | 8.61 | 48.46 | 12.39 | 6.64 | 99.12 | 40.93 | 46.58 |
| *P* | 0.004 | <0.001 | 0.003 | 0.014 | <0.001 | <0.001 | <0.001 |

Note. *G*-tests evaluate whether outcross pollen sires an equivalent number of seeds as self pollen when equal amounts of both pollen types are present on the stigma. Offspring were identified as selfed or outcrossed on the basis of their genotype at one of two allozyme loci. TPI = trioephosphate isomerase, MDH = malate dehydrogenase.

## Table 3

**ANOVA to Determine the Effect of Pollen Type on the Length of Pollen Tubes in *Campanulastrum americanum***

| Source of variation | df | F/Z | P |
|---|---|---|---|
| Maternal plant | | 2.67 | <0.004 |
| Pollen type | 1, 99 | 0.99 | <0.323 |
| Time interval | 3, 99 | 107.80 | <0.001 |
| Type × interval | 3, 99 | 0.23 | <0.877 |

Note. Self and outcross pollen tubes were measured 2, 3.5, 5, and 24 h after pollination. A $Z$ value is reported for random effects (maternal plant).

so that pollen tubes would be visible. The two halves of the style were mounted on one slide and moistened with 50% ethanol. A cover slip was placed over the styles and sealed at the edges with petroleum jelly followed by clear nail polish to preserve the slides for later viewing.

Pollen tube growth was quantified by measuring length and counting number of pollen tubes. The aniline blue stain made the walls of the pollen tubes fluoresce green under an epifluorescence microscope, enabling tracking and counting of pollen tubes. Pollen tubes tended to grow in clusters down the style, so we measured the length from the stigma to the end of the cluster. The lengths of the clusters in the two halves of each style were averaged. To complement the pollen tube length measurements, we counted the number of pollen tubes in each of four regions from the tip to the base of the style. Four equal sections of the style were marked on the cover slip, starting from the base of the stigma. We counted the number of pollen tubes at the end of each quarter of the style using a fluorescence microscope at ×200 magnification. Counts from the two halves of the style were averaged.

Pollen tube lengths and counts were analyzed using ANOVA. The analysis of pollen tube length used time since pollination and pollen type as fixed effects and maternal plant as a random factor (PROC MIXED; SAS Institute 2000). Pollen tube length was square root transformed. Pollen tube count was analyzed using a repeated-measures ANOVA that included section of the style, pollen type, and time since pollination as fixed effects and maternal plant as a random factor. For some individuals, the first and second sections of the 24-h time interval styles were dropped from analysis because the pollen tubes in these sections had begun to degrade.

## Results

### *Floral Display Surveys in a Natural Population*

Selfing-capable plants were common in the population surveyed. Plants were considered capable of geitonogamous self-pollination if they had at least one male-phase flower and one female-phase flower open simultaneously. Averaged over sample dates, approximately half the plants sampled were capable of selfing (table 1). Between 29% and 66% of plants exhibited potential for selfing, peaking at the middle survey date. Selfing-capable plants had an average of eight flowers open. Average display size varied over survey dates by around five flowers (table 1). Selfing-capable plants had an average of 4.3 open female-phase flowers and 2.7 open male-phase flowers (fig. 1). For each survey, distance to the nearest source of outcross pollen (average of three closest neighbors) was calculated as the median distance for all selfing-capable plants. Only selfing-capable plants were used in this calculation because the probability of selfing may be influenced by distance to outcross pollen. Some plants were only a few centimeters from another plant, while a few were several meters from the nearest neighboring plant. The median distance to neighbors was calculated for each sample date because a few isolated plants skewed the average distance. Median distance varied by only a few centimeters across sample dates (table 1).

### *Siring Success of Self and Outcross Pollen*

Proportions of selfed and outcrossed progeny differed significantly from the 50 : 50 ratio applied to the stigmas. Outcrossed offspring outnumbered selfed offspring by an average of four to one (fig. 2). The percentage of selfed progeny ranged from 6% to 34% across the maternal plants. For all maternal plants, the proportion of outcrossed progeny was significantly greater than would be expected if self and outcross pollen had equal siring ability in the 50-50 pollen loads (table 2). The number of seeds did not differ between fruits from flowers that received either self (50.1 ± 2.9) or outcross pollen (47.4 ± 4.1; $F_{1,49} = 0.14$, $P = 0.71$).

### *Pollen Tube Growth Rates*

There was no significant difference in the growth rates of self and outcross pollen tubes (table 3; fig. 3). At each time interval, the two types of pollen had grown to approximately the same point. As expected, the time interval at which the style was collected significantly affected the length of the pollen tube cluster. At 5 h, both self and outcross pollen tubes had grown to about halfway down the style, and by 24 h they had grown down the entire length of the style. The identity of the maternal plant also significantly influenced the pollen tube lengths.

Pollen tube counts in the four style sections revealed no overall difference in growth rates of self and outcross pollen tubes (table 4; fig. 4). Counts for self and outcross pollen tubes also did not differ over time or with section of the

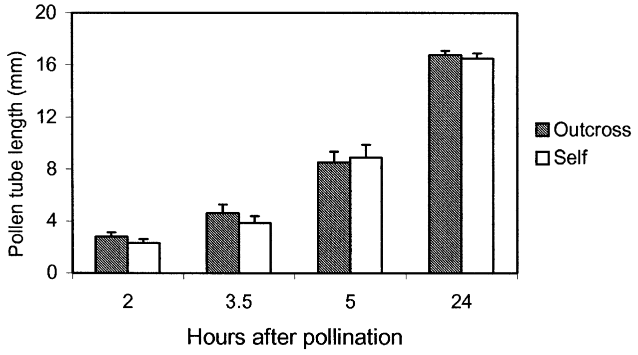

**Fig. 3** Length and SE of self and outcross pollen tubes in *Campanulastrum americanum*. The longest groups of pollen tubes in each style were measured at four time intervals after pollination.

## Table 4

**Repeated-Measures ANOVA to Determine the Effect of Pollen Type on Pollen Tube Counts for Different Sections of *Campanulastrum americanum* Styles**

| Source | df | *F*/*Z* | *P* |
|---|---|---|---|
| Maternal plant | | 16.56 | <0.001 |
| Style section | 3, 90 | 26.17 | <0.001 |
| Pollen type | 1, 27 | 1.38 | <0.250 |
| Time | 3, 29 | 60.30 | <0.001 |
| Section × type | 3, 80 | 0.42 | <0.737 |
| Section × time | 9, 87 | 13.12 | <0.001 |
| Type × time | 3, 27 | 1.40 | <0.263 |
| Section × type × time | 9, 81 | 0.31 | <0.969 |

Note. Styles were divided into four equal sections from the stigma to the base of the style. The numbers of self and outcross pollen tubes in each section were counted 2, 3.5, 5, and 24 h after pollination. A *Z* value is reported for random effects (maternal plant).

style. As expected, the number of pollen tubes depended on style section, and the influence of section on pollen tube counts changed with time since pollination. The maternal plant also had a significant effect on pollen tube counts.

## Discussion

Floral display characteristics of *Campanulastrum americanum* suggest high potential for within-plant pollen transfer in nature. Approximately one-half of all plants surveyed at a given point in time were capable of self-pollination. Furthermore, there were typically more flowers open in female phase than in male phase. Thus, if a pollinator visited those female-phase flowers after landing on a male-phase flower, it could carry self pollen to multiple female-phase flowers. The selfing-capable plants surveyed had on average eight flowers open. Earlier work demonstrated that in plants with nine open flowers, around half of bee visits could result in geitonogamy (Galloway et al. 2002). Since plants typically received around eight pollinator visits in 15 min (Galloway et al. 2002), plants surveyed could be receiving 16 geitonogamous visits per hour. In this population, the frequency of bumblebee pollinator visits to male- and female-phase flowers and the gender of initial flower visited in a cluster do not differ from expectations based on the gender ratio of the plant (Galloway et al. 2002), suggesting that display structure does not reduce the potential for geitonogamy. Although self pollen produces an approximately equal number of seeds that readily germinate, a recent study found that in pots, selfed offspring suffer a 26% reduction in cumulative fitness because of inbreeding depression (Galloway et al. 2003), with a substantially greater fitness reduction in nature (L. F. Galloway and J. R. Etterson, unpublished manuscript). Given the frequent contact with self pollen and inbreeding depression, selection would be expected to favor any reduction in self-fertility.

Distance to the nearest sources of outcross pollen was fairly small. If distance between plants, is small, pollinators may frequently move between plants, facilitating outcrossing. Observation of pollinators in *C. americanum* has shown that as the distance between a plant and its nearest neighbors increases, the number of pollinators decreases while both the number of flowers visited on a foraging bout and the number of potentially geitonogamous visits increases (Galloway et al. 2002). For plants less than 50 cm from their nearest neighbors, on average half of the foraging bouts result in geitonogamous pollination. In contrast, foraging bouts to plants with intermediate (51–100 cm) and more distant neighbors (101–500 cm) results in an average of 1.18 and 1.34 geitonogamous visits, respectively (L. F. Galloway, unpublished data). Similar patterns of pollinator behavior have been found in other systems (e.g., Klinkhamer et al. 1989; Klinkhamer and de Jong 1990; Karron et al. 1995; Mustajarvi et al. 2001). Therefore, in this population, the relatively small distances between plants may enhance the potential for outcrossing.

Pollinations containing equal mixtures of self and outcross pollen produced a majority of outcrossed progeny. An earlier study of inbreeding depression using individuals from the same *C. americanum* population grown under the same conditions found no significant difference between self (80% ± 5%) and outcross (82% ± 3%) germination (Galloway et al. 2003) or juvenile survivorship (eight self vs. six outcross died, 4% mortality; L. J. Kruszewski and L. F. Galloway, unpublished data). The excess of outcrossed progeny is therefore not likely to be due to lack of self-seed germination or seedling mortality. The high proportion of outcrossed progeny strongly suggests cryptic self-incompatibility, a decreased production of self seed. Given the likelihood of receiving self pollen and the viability of self seeds, cryptic self-incompatibility must act after pollination and before ovule fertilization.

Differential pollen tube growth is a common mechanism of cryptic self-incompatibility (e.g., Bowman 1987; Hessing 1989; Weller and Ornduff 1989; Aizen et al. 1990; Cruzan

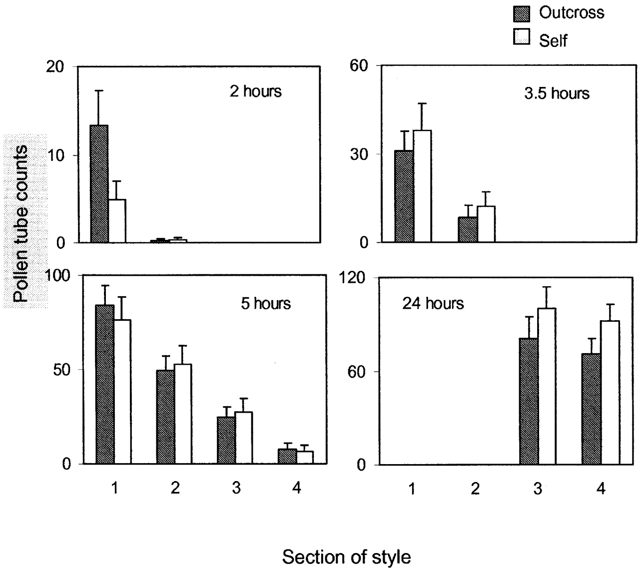

**Fig. 4** Outcross and self pollen tube counts and SE in *Campanulastrum americanum*. The numbers of pollen tubes in each section of the style were counted at four time intervals after pollination. Section 1 shows the number of pollen tubes having reached the end of the first quarter of the style from the stigma. Section 4 shows the number of pollen tubes that have reached the base of the style.

and Barrett 1996; Eckert and Allen 1997). However, we found self and outcross pollen tube lengths and counts were similar, suggesting that self and outcross pollen tubes grow at the same rates. Therefore, in *C. americanum*, cryptic self-incompatibility appears to manifest itself in an unusual way. One possibility is that outcross pollen and self pollen differ in germination rate or in pollen tube attrition (Thomson 1989; Weller and Ornduff 1989; Montalvo 1992; Cruzan and Barrett 1993; Rigney et al. 1993; Smith-Huerta 1996; Skogsmyr and Lankinen 2002). A higher percentage of outcross pollen germination or lower pollen tube attrition would increase the odds of ovule fertilization. However, the pollen tube counts should have detected any differences in germination or pollen tube attrition; therefore, these mechanisms are unlikely. A further study in which pollen load is varied along with pollen mixture would directly address this possibility (e.g., Cruzan and Barrett 1996). Another possibility is that outcross pollen outperforms self pollen later, once the pollen tubes have entered the ovary. If self pollen tubes are delayed from fertilizing ovules, outcross pollen tubes may have a seed-siring advantage.

Alternatively, cryptic self-incompatibility in *C. americanum* may manifest itself only when both self and outcross pollen are present in the pistil. Outcross pollen could prevent germination of self pollen on the stigma, hinder self pollen tubes growing down the style, or prevent self pollen from fertilizing ovules (Hessing 1989; Thomson 1989; Aizen et al. 1990; Cruzan and Barrett 1993; Snow and Spira 1993; Marshall et al. 1996; Marshall and Diggle 2001). Interference competition, as such mechanisms are termed, is often greatest when pollen grains are thoroughly mixed and come into direct contact with each other, suggesting a chemical basis for the interaction (Pasonen and Käpyla 1998; Nemeth and Smith-Huerta 2002; Skogsmyr and Lankinen 2002). For example, in *Clarkia unguiculata*, pollen loads containing part self and part outcross pollen had significantly lower germination rates and slower pollen tube growth early in the style than did pure outcross loads (Nemeth and Smith-Huerta 2002). In *Dianthus chinensis*, slight intrinsic differences between self and outcross pollen tube growth rates increased greatly when both pollen types were present in the same pistil (Aizen et al. 1990). The maternal plant may also mediate pollen-pollen interactions. As the pollen tube growth study showed, the maternal plant significantly influences the growth rates of pollen tubes in the style. The maternal plant may regulate pollen tube growth by controlling nutrients available to specific pollen types during pollen germination or tube growth in the style (Bertin 1985; Marshall and Folsom 1991; Baker and Shore 1995; Marshall and Diggle 2001; Skogsmyr and Lankinen 2002). Pistil-mediated interactions may also occur in the ovary before fertilization (Marshall and Diggle 2001; Nemeth and Smith-Huerta 2002).

The results of this study reveal that cryptic self-incompatibility is present in this population of *C. americanum*. The presence of cryptic self-incompatibility likely reflects conflicting needs to produce selfed offspring and avoid inbreeding depression. The mechanism that favors outcross pollen may have evolved from selection pressure of frequent mixed self and outcross pollinations combined with later-life inbreeding depression. However, the ability of self pollen to produce viable and abundant seeds may reflect an advantage to preserving self-compatibility. Delayed selfing as seen in related *Campanula rapunculoides* and other taxa, similarly, is a mechanism of reproductive assurance (e.g., Vogler et al. 1998; Kalisz et al. 1999; Goodwillie et al. 2004). Since *C. americanum* populations frequently contain fewer than 50 flowering individuals and isolated plants are common (personal observation), outcross pollen may not be consistently available. Polyploidy is often linked to a breakdown in physiological self-incompatibility caused by new allelic interactions in pollen (de Nettancourt 2001; Galloway et al. 2003; but see Mable 2004). Loss of self-incompatibility may select for alternative mechanisms to reduce selfing in autotetraploids such as *C. americanum*.

Cryptic self-incompatibility enables individuals to maximize fitness in varying reproductive situations by ensuring reproduction in the absence of outcross pollen yet favoring outcrossing whenever possible. Cryptic self-incompatibility could provide an advantage to species that experience a combination of mixed self and outcross pollinations, varying availability of outcross pollen, and inbreeding depression.

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## Literature Cited

Aizen MA, KB Searcy, DL Mulcahy 1990 Among- and within-flower comparisons of pollen tube growth following self and outcross pollinations in *Dianthus chinensis*. Am J Bot 77:671–676.

Baker HG 1966 The evolution, functioning, and breakdown of heteromorphic incompatibility systems. I. The Plumbaginaceae. Evolution 20:349–368.

Baker HG, JS Shore 1995 Pollen competition in *Turnera ulmifolia* (Turneraceae). Am J Bot 82:717–725.

Barrett SCH, CG Eckert 1992 Variation and evolution of plant mating systems. Pages 229–254 *in* S. Kawano, ed. Biological approaches and evolutionary trends in plants. Academic Press, San Diego, CA.

Bateman AJ 1956 Cryptic self-incompatibility in the wallflower: *Cheiranthus cheiri*. Heredity 10:257–261.

Bertin RI 1985 Nonrandom fruit production in *Campsis radicans*: between-year consistency and effects of prior pollination. Am Nat 126:750–759.

Bowman RN 1987 Cryptic self-incompatibility and the breeding system of *Clarkia unguiculata* (Onagraceae). Am J Bot 74: 471–476.

Casper BB, LS Sayigh, SS Lee 1988 Demonstration of cryptic incompatibility in distylos *Amsinckia douglasiana*. Evolution 42: 248–253.

Charlesworth D, B Charlesworth 1987 Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18:237–268.

Cruzan MB, SCH Barrett 1993 Contribution of cryptic self-incompatibility to the mating system of *Eichornia paniculata* (Pontederiaceae). Evolution 47:925–934.

——— 1996 Postpollination mechanisms influencing mating patterns and fecundity: an example from *Eichhornia paniculata*. Am Nat 147:576–598.

Darwin C 1876 The effects of cross and self fertilization in the vegetable kingdom. J Murray, London.

——— 1877 The different forms of flowers on plants of the same species. J Murray, London.

de Nettancourt D 2001 Incompatibility in angiosperms. Springer, Berlin.

Eckert CG, M Allen 1997 Cryptic self-incompatibility in tristylous *Decodon verticillatus* (Lythraceae). Am J Bot 84:1391–1397.

Eckert CG, SCH Barrett 1994 Post-pollination mechanisms and the maintenance of outcrossing in self-compatible *Decodon verticillatus*. Heredity 72:396–411.

Evanhoe L, LF Galloway 2002 Floral longevity in *Campanula americana* (Campanulaceae): a comparison of morphological and functional gender phases. Am J Bot 88:832–840.

Galloway LF, T Cirigliano, K Gremski 2002 The contribution of display size and dichogamy to potential geitonogamy in *Campanula americana*. Int J Plant Sci 163:133–139.

Galloway LF, JR Etterson, JL Hamrick 2003 Outcrossing rate and inbreeding depression in the herbaceous tetraploid *Campanula americana*. Heredity 90:308–315.

Glover DE, SCH Barrett 1986 Variation in the mating system of *Eichornia paniculata*. Evolution 40:1122–1131.

Goodwillie C, KL Partis, JW West 2004 Transient self-incompatibility confers delayed selfing in *Leptosiphon jepsonii* (Polemoniaceae). Int J Plant Sci 165:387–395.

Hessing MB 1989 Differential pollen tube success in *Geranium caespitosum*. Bot Gaz 150:404–410.

Johnston MO 1993 Tests of two hypotheses concerning pollen competition in a self-compatible, long-styled species (*Lobelia cardinalis*: Lobeliaceae). Am J Bot 80:1400–1406.

Jones KN 1994 Nonrandom mating in *Clarkia gracilis* (Onagraceae): a case of cryptic self-incompatibility. Am J Bot 81:195–198.

Kalisz SJ, D Vogler, B Fails, M Finer, E Shepard, T Herman, R Gonzales 1999 The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). Am J Bot 86:1239–1247.

Karron JD, NN Thumser, R Tucker, AJ Hessenauer 1995 The influence of population density on outcrossing rates in *Mimulus ringens*. Heredity 75:175–180.

Kearns CA, DW Inouye 1993 Techniques for pollination biologists. University Press of Colorado, Niwot.

Klinkhamer PGL, TJ de Jong 1990 Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Eichum vulgare* (Boraginaceae). Oikos 57:399–405.

Klinkhamer PGL, TJ de Jong, G-J de Bruyn 1989 Plant size and pollinator visitation in *Cynoglossum officinale*. Oikos 54:201–204.

Lande R, DW Schemske 1985 The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 40:388–404.

Lewis D 1979 Sexual-incompatibility in plants. Edward Arnold, London.

Lloyd DG 1992 Self- and cross-fertilization in plants. II. The selection of self-fertilization. Int J Plant Sci 153:370–380.

Lloyd DG, CJ Webb 1986 The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. N Z J Bot 24:135–162.

Mable BK 2004 Polyploidy and self-compatibility: is there an association? New Phytol 162:803–811.

Marshall DL, PK Diggle 2001 Mechanisms of differential pollen donor performance in wild radish, *Raphanus sativus*. Am J Bot 88: 242–257.

Marshall DL, MW Folsom 1991 Mate choice in plants: an anatomical to population perspective. Annu Rev Ecol Syst 22:37–63.

Marshall DL, MW Folsom, C Hatfield, T Bennett 1996 Does interference competition among pollen grains occur in wild radish? Evolution 50:1842–1848.

Montalvo AM 1992 Relative success of self and outcross pollen comparing mixed- and single-donor pollinations in *Aquilegia caerulea*. Evolution 46:1181–1198.

Mustajarvi K, P Siikamaki, S Rytkonen, A Lammi 2001 Consequences of plant population size and density for plant-pollinator interactions and plant performance. J Ecol 89:80–87.

Nemeth BM, NL Smith-Huerta 2002 Effects of pollen load composition and deposition pattern on pollen performance in *Clarkia unguiculata*. Int J Plant Sci 163:795–802.

Pasonen HL, M Käpyla 1998 Pollen-pollen interactions in *Betula pendula in vitro*. New Phytol 138:481–487.

Richards AJ 1986 Plant breeding systems. George Allen & Unwin, London.

Rigney LP, JD Thomson, MB Cruzan, J Brunet 1993 Differential success of pollen donors in a self-compatible lily. Evolution 47: 915–924.

Routley MB, BC Husband 2003 The effect of protandry on siring success in *Chamerion angustifolium* with different inflorescence sizes. Evolution 57:240–248.

SAS Institute 2000 SAS/STAT, version 8.0. SAS Institute, Cary, NC.

Skogsmyr I, A Lankinen 2002 Sexual selection: an evolutionary force in plants. Biol Rev 77:537–562.

Smith-Huerta NL 1996 Pollen germination and tube growth in selfing and outcrossing populations of *Clarkia tembloriensis* (Onagraceae). Int J Plant Sci 157:228–233.

Snow AA, TP Spira 1993 Differential pollen tube growth rates and non-random fertilization in *Hibiscus moscheutos* (Malvaceae). Am J Bot 78:1419–1426.

Soltis DE, CH Haufler, DC Darrow, GJ Gastony 1983 Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am Fern J 73:9–27.

Thomson JD 1989 Germination schedules of pollen grains: implications for pollen selection. Evolution 43:220–223.

Uyenoyama MK 1986 Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. Evolution 40:388–404.

Vogler D, C Das, A Stephenson 1998 Phenotypic plasticity in the expression of self-incompatibility in *Campanula rapunculoides*. Heredity 81:546–555.

Weller SG, R Ornduff 1977 Cryptic self-incompatibility in *Amsinckia grandiflora*. Evolution 31:47–51.

——— 1989 Incompatibility in *Amsinckia grandifolia* (Boraginaceae). Am J Bot 78:801–804.

Wendel JF, R Parks 1982 Genetic control of isozymes variation in *Camellia japonica* (Theaceae). J Hered 73:197–204.