SELF-Sterility in the Understory Herb Clintonia borealis (Liliaceae)

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In the understory perennial Clintonia borealis, self-pollination yields lower seed number per fruit than cross-pollination. To measure the magnitude of this partial self-sterility and identify the pre- and postzygotic mechanisms on which it is based, we conducted self- and outcross-pollinations in 34 patches in a natural population and estimated the effects on seed and fruit production, frequency of fertilization, and postzygotic endosperm development. Plants in nine of the 34 patches set no fruit after pollination. In the remaining patches, 22% of self-pollinated flowers and 54% of outcrossed flowers set fruit. Mean seed set per fruit was 0.38 (SE = 0.04) for outcrossed flowers compared with 0.09 (SE = 0.04) after self-pollination. The self-outcross ratio of seed set ranged from 1.88 to 0.0 among patches, but the effects of selfing did not differ significantly among patches. Observations of cleared ovules from 1, 2, 3, 4, 6, 8, and 10 d after pollination showed that fertilization does not occur until day 3. The proportion of ovules fertilized increased with time and, at 10 d, was 0.44 (SE = 0.18) for outcross-pollinations and 0.14 (SE = 0.06) for selves. In addition, the number of endosperm nuclei in fertilized ovules increased more rapidly and the size of full seeds was larger after outcross-pollination than self-pollination. Our results indicate that prezygotic mechanisms preventing fertilization can account for much of the self-sterility observed in C. borealis in the field. However, some postzygotic differences were also evident, which may influence the number and size of selfed versus outcrossed offspring.

Keywords: reproductive biology, self-sterility, partial incompatibility, fertilization frequency, prezygotic, postzygotic, endosperm nuclei, inbreeding depression.

Introduction

Most flowering plants are hermaphroditic and thus potentially self-pollinating (Fryxell 1957; Richards 1997). Despite the opportunities for self-fertilization, many plant species are completely or partially self-sterile, producing few or no offspring after self-pollination (Darwin 1876; de Nettancourt 1977, 1997). Self-sterility has important implications for the design of breeding programs and cultivation practices for domesticated plants, and it represents one of the most important selective forces governing the evolution of pollination systems in natural populations. However, the underlying proximal causes of self-sterility and their evolutionary origins are still poorly understood (Seavey and Bawa 1986; Barrett 1988; Husband and Schemske 1996).

The genetic causes of self-sterility can be divided into two categories: physiological self-incompatibility and inbreeding depression. In systems of self-incompatibility (SI), self-pollen, or pollen sharing the same incompatibility alleles as occur in the pollen recipient are recognized and rejected on the stigma, in the style, or in the ovary of the maternal parent (Brock 1954; Seavey and Bawa 1986; Barrett 1988; Sage et al. 1994). However, some cases of postzygotic self-sterility have been interpreted as late acting, or ovarian SI (Seavey and Bawa 1986; Sage and Williams 1991; Sage et al. 1994). Inbreeding depression, on the other hand, is an expression of the genetic constitution of the zygote. Reduced fitness of selfed offspring due to inbreeding depression generally results from increased homozygosity and the expression of deleterious, recessive alleles (Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Husband and Schemske 1996). Although the two mechanisms of self-sterility are conceptually distinguishable, they are sometimes difficult to distinguish empirically, particularly early-acting inbreeding depression and late-acting SI. In most cases, identifying such post- and prezygotic processes requires direct observation of pollen tube growth, fertilization events, or embryo development (Seavey and Bawa 1986; Seavey and Carter 1994, 1996; Manicacci and Barrett 1996).

Although the immediate outcomes of both SI and inbreeding depression are to reduce the number of selfed offspring, these processes have different fitness consequences for the maternal plant. Active rejection of self-pollen in the stigma or style, as observed in self-incompatibility, may occur sufficiently early so that self-pollination would not reduce the number of ovules available for cross-fertilization (but see Waser and Price 1991; Scribalo and Barrett 1994). In contrast, postzygotic inbreeding depression is potentially more costly to the maternal parent in that ovules are usurped by self-fertilization and energy “wasted” on the early development of selfed zygotes (Lewis 1979; Beach and Kress 1980; Barrett et al. 1996). Alternatively, it has been suggested that postponing the blockage of self-pollen may increase fitness by extending the period of time over which pollen genotypes may be “evaluated” by the maternal parent (Seavey and Bawa 1986). The contrasting implications of pre- and postzygotic self-sterility are related not

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only to the timing of their effects but also to the mechanisms for their origin. Whereas inbreeding depression is the genetic by-product of mutation and inbreeding on zygote fitness, incompatibility is a prezygotic mechanism that may have evolved as a response to inbreeding depression (Charlesworth and Charlesworth 1979).

The reproductive biology of the understory perennial *Clintonia borealis* has been examined in numerous studies, and, in all cases, it has exhibited low seed set upon self-pollination compared with cross-pollination (Galen et al. 1985; Galen and Weger 1986; Barrett and Helenurm 1987). Barrett and Helenurm (1987) found that self-pollination resulted in 57% fewer seeds per fruit than cross-pollination, and Galen et al. (1985) reported self-fertility as one-third of that from outcrossing. Moreover, seed production upon selfing appears to be highly variable, with some individuals exhibiting complete self-fertility and others, complete sterility (Galen et al. 1985; Galen and Weger 1986). This apparent quantitative variation in fertility may be the result of inbreeding depression caused by variation in genetic load among individual clones. Alternatively, systems of partial self-incompatibility may exhibit similar patterns of seed production (Cooper and Brink 1940; Waser 1993).

Our goal was to estimate the magnitude of self-sterility in a natural population of *C. borealis* and to examine the roles of pre- and postzygotic mechanisms. To address this, we conducted cross- and self-pollinations on 34 clones in a southern Ontario population and examined seed set, frequency of fertilization, and endosperm development. Specifically, we addressed the following questions: Do seed set and seed size differ between offspring from self- and cross-pollination? Does the degree of self-sterility, measured as the relative production of selfed seeds, vary among clones within a population? Are there differences in rates of fertilization between self- and cross-pollinations? Finally, are there differences in the rate of postzygotic endosperm development between self- and cross-pollinations?

**Material and Methods**

**Natural History and Study Site**

*Clintonia borealis* (Ait.) Raf. (Liliaceae) is a perennial, forest understory herb found throughout the northeastern United States and Canada (Fernald 1950). It forms long-lived clones, the ramets of which are connected via rhizomes (Pitelka et al. 1985). Ramets live for 1 yr, and those that bloom produce from one to six weakly protogynous yellow-green flowers (*Ga-*len and Weger 1986). Pollination is performed primarily by bumblebees (Galen et al. 1985; Barrett and Helenurm 1987). Barrett and Helenurm (1987) found that self-pollination resulted in 57% fewer seeds per fruit than cross-pollination, and Galen et al. (1985) reported self-fertility as one-third of that from outcrossing. Moreover, seed production upon selfing appears to be highly variable, with some individuals exhibiting complete self-fertility and others, complete sterility (Galen et al. 1985; Galen and Weger 1986). This apparent quantitative variation in fertility may be the result of inbreeding depression caused by variation in genetic load among individual clones. Alternatively, systems of partial self-incompatibility may exhibit similar patterns of seed production (Cooper and Brink 1940; Waser 1993).

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To examine the effects of selfing on fruit and seed production, we hand-pollinated flowers in the field in 1994. Because of the difficulty in finding inflorescences with sufficient numbers of flowers, we replicated the hand-pollination treatments on a pair of neighboring inflorescences within each patch. Prior to flowering, pairs of inflorescences separated by <0.5 m were located within 34 patches in the population and covered with mesh bags to exclude pollinators. Two flowers on each inflorescence within a pair were randomly assigned one of two pollination treatments: self-pollination and outcross-pollination. For self-pollinated flowers, pollen was acquired from another within the target flower or from another flower on the same inflorescence. Pollen for the outcross treatment was gathered from two flowers at least 10 m away from each other and the target flower. Our choice of a minimum distance of 10 m was based on findings by Galen et al. (1985), who showed no difference in seed set between outcross-pollinations with pollen collected 10 or 200 m away.

A third flower on each of the paired inflorescences was used for two different control pollinations. On one randomly chosen inflorescence of the pair, the third flower was left unpollinated to check for inadvertent pollen transfer. On the remaining inflorescence, the flower was pollinated using pollen from the other inflorescence in the pair to confirm that the paired inflorescences belonged to the same genet. If the two inflorescences were ramets from the same genet, as the electrophoretic screening indicated, seed set in this treatment should not differ from the self-pollination treatment. All treatments were randomly assigned to the first three flowers within an inflorescence. Excess flowers were removed immediately after performing the last pollination. Flowers were emasculated prior to all non-self-pollinations. In addition to the inflorescences chosen for hand pollinations, 34 inflorescences, one near each experimental pair, were tagged and left to be open pollinated.

Following fruit maturation, berries were harvested, and the number of full seeds and ovules within each berry were counted. A berry was classified as a fruit if it had at least one full seed. Full seeds were readily identified as they were larger and plumper than undeveloped or unfertilized ovules and had a well-developed seed coat. Seed set per fruit was measured as the number of full seeds relative to the total number of ovules produced. Differences in seed set among self-, outcross-, and control pollinations were analyzed using a two-way, mixed-model ANOVA with patch membership (random effect) and pollination treatment (fixed effect) as main effects. Means for the pollination treatments were compared using a Tukey-Kramer HSD test. Only patches

**Fruit and Seed Production**
with replicate values for each treatment were used in the analysis. Results based on arcsine-transformed data were the same as for untransformed data, so the latter are presented here. In addition, seed set in unpollinated flowers (controls) was compared to zero using a one-tailed t-test. Finally, to determine the effect of pollination on postzygotic development, we measured the length and width of all full seeds within the fruits from the experimental pollinations and compared their means using a Tukey-Kramer HSD test.

Frequency of Fertilization and Endosperm Development

To examine whether self-sterility was associated with pre- or postzygotic factors, we compared the frequency of fertilization of ovules and endosperm development in zygotes after self- and cross-pollination. Seventy-one flowering ramets with at least three flowers were chosen in 1995; on each, flowers were randomly assigned one of three pollination treatments: outcross-pollen, self-pollen, and no pollen. Pollinations,emasculations, and removal of extra flowers were performed as in the previous year.

To examine temporal variation in fertilization, 10 ovaries were collected at 1, 2, 3, 4, 6, 8, and 10 d following pollination. Each inflorescence was randomly assigned to one of these harvest times. Upon collection, fruits were fixed in 50% FAA and transferred to 50% ethanol after 24 h. Ovules were cleared for light microscopy following methods in Stelly et al. (1984), with modifications used by Scribailo and Barrett (1994). Each ovary was opened and ovules were placed on a glass slide in methyl salicylate and then examined under 100× magnification using a Leica DMRB Brightfield microscope.

We examined all ovules in 6–10 fruits per pollination treatment (self and outcross) from each harvest date (total n = 2055 ovules) under the microscope and classified them as unfertilized or fertilized. Development of the embryo sac in C. borealis is tetrasporic. The mature embryo sac is 5–6 nucleate composed of an egg cell, two synergids, one upper polar nucleus, and one or two abortive nuclei at the chalazal end. The lower polar nucleus is absent, and no antipodal cells are formed (Walker 1944; Pahuja and Kumar 1971). Ovules were classified as fertilized when cell divisions in the polar nucleus and egg nucleus were observed. In some ovules in which the egg cell could not be observed clearly, divisions of the polar nucleus were assumed to indicate double fertilization.

To determine whether selfed and outcrossed embryos differed in their early development, we estimated the number of endosperm nuclei in all observed fertilized ovules. Postfertilization endosperm development was scored by counting numbers of endosperm nuclei in fertilized ovules. Differences in fertilization frequency and postfertilization endosperm development between self- and outcross-pollination treatments were investigated using a Model I, two-way ANOVA with pollination type and elapsed time since pollination as main effects. No fertilizations or endosperm development were observed in unpollinated flowers, and therefore unpollinated flowers were not included in the following analyses. Fertilization frequencies were arcsine transformed before analysis; however, untransformed data are presented in the “Results.” All statistical analyses were conducted using JMP 3.0 (SAS Institute 1994) statistical software.

Results

Fruit and Seed Production

Of the 34 patches used for experimental pollinations in 1994, three were damaged prior to fruit set and thus were excluded from further analyses. In 25 of the remaining 31 patches, fruit set occurred on at least one of the two ramets. Across the 25 patches, mean fruit set was 0.22 (SE = 0.05) for self-pollinated flowers and 0.54 (SE = 0.06) for outcross-pollinated flowers. Mean fruit set in unpollinated controls was 0.02 (SE = 0.03).

On average, Clintonia borealis fruits contained 12.5 (SE = 0.30) ovules, yet in unpollinated control flowers, seed set averaged 0.01 (SE = 0.06). Seeds were produced in only one of the control flowers, and the mean was not significantly different from zero in a one-tailed t-test (t = 1.0, df = 25, P = 0.16). The ANOVA on seed set indicated highly significant differences among pollination treatments and marginally significant differences among patches (table 1). Mean seed set for intrapatch-pollination (mean = 0.19, SE = 0.06) was approximately twice that from self-pollination (mean = 0.09, SE = 0.04); however, there was no significant difference between these treatments (Tukey-Kramer HSD, Q = 2.8, P > 0.05; fig. 1). Seed set per fruit from outcross-pollinations was more than four times higher (mean = 0.38, SE = 0.04) than in self-pollinations (fig. 1). Open-pollinated seed set averaged 0.45 (SE = 0.05), significantly higher than all but outcross-pollinations. Mean seed set ranged from 0 to 0.94 per patch for outcrosses and from 0 to 0.89 for self-pollinations (fig. 2). Fifteen of the 25 patches that produced at least one fruit had no selfed fruit. For those patches with selfed and outcrossed fruit, the self : outcross ratio of seed set varied from 1.88 to 0.10 among patches. Despite this wide variation, the interaction between pollination treatment and patch was not significant (table 1).

Seed size, calculated as the product of seed length and width, differed significantly among pollination treatments. Self- and intrapatch-pollinations produced seeds of similar size (self mean = 0.44 mm², SE = 0.02; intrapatch mean = 0.40 mm², SE = 0.03). Outcrossed pollinations produced significantly higher mean seed size (0.54 mm², SE = 0.01) than self- or intrapatch-pollinations (mean = 0.36 mm², SE = 0.02; fig. 3).

Table 1

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patch</td>
<td>4.30</td>
<td>30</td>
<td>1.79</td>
<td>0.06</td>
</tr>
<tr>
<td>Pollination type</td>
<td>1.88</td>
<td>1</td>
<td>23.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Patch × pollination type</td>
<td>2.40</td>
<td>30</td>
<td>1.10</td>
<td>0.38</td>
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<tr>
<td>Residual</td>
<td>3.19</td>
<td>44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. The main effect “patch” tests for variation in seed production among 31 patches; “pollination type” tests for the effects of outcross- vs. self-pollinations. Patch was treated as a random effect, pollination type as a fixed effect. Means for pollination type are presented in fig. 1.
Fig. 1 Mean (±SE) seed set after outcross-, self-, intrapatch self-, control, and open pollinations for 31 patches of *Clintonia borealis*. Statistical analysis of these data is presented in table 1. Results of Tukey-Kramer HSD multiple comparison are shown; treatments sharing the same lowercase letter were not significantly different.

Fig. 2 Mean seed set for selfed and outcrossed flowers in 25 patches of *Clintonia borealis*. Patches are ordered by the magnitude of self : outcross ratio of seed set per patch.

Discussion

The magnitude of self-sterility observed in this study was greater than in previous investigations of *Clintonia borealis* (Galen et al. 1985; Barrett and Helenurm 1987). Seed set upon selfing was 0.09 in our study, which is <25% of outcrossed seed set (0.38) and less than half the estimates of selfed seed set (0.22 and 0.32) reported by Barrett and Helenurm (1987) and Galen et al. (1985), respectively. These differences in the strength of self-sterility may reflect variation in both environmental and genetic determinants of seed production among populations (Lee 1988). Differences among populations in the impact of self-pollination may arise because of variation in composition of incompatibility alleles and differences in the strength of the incompatibility reaction among those alleles. Variation in genetic load may also be important, particularly among populations that differ in inbreeding history as influenced by mating system, pollination, population size, and colonization history (Schemske and Lande 1985). These genetic factors may operate singly or in combination to account for variation in seed set upon selfing among populations of *C. borealis*.

Environmental differences, such as variation in resource and pollen availability, may also be contributing to interpopulation variation in self-sterility. High fruit and seed set in open-pollinated flowers indicate that pollen is not limited in *C. borealis* and that there is a sufficient amount of outcross-pollen for adequate seed set. This is supported by Galen et al. (1985), who showed that the addition of pollen to open-pollinated stigmas of *C. borealis* did not increase seed set. Similarly, Barrett and Helenurm (1987) found no evidence of pollen limi-
Table 2

ANOVA for Frequency of Fertilization and the Number of Endosperm Nuclei Observed in Fertilized Ovules of Clintonia borealis

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Fertilization frequency</th>
<th>No. of endosperm nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollination</td>
<td>1</td>
<td>14.94***</td>
<td>7.37*</td>
</tr>
<tr>
<td>Day</td>
<td>4</td>
<td>6.54***</td>
<td>13.52***</td>
</tr>
<tr>
<td>Pollination × day</td>
<td>4</td>
<td>4.81***</td>
<td>12.67***</td>
</tr>
</tbody>
</table>

Note. The values presented are F-ratios from a two-way, Model I ANOVA. Flowers were hand pollinated using either self- or outcross-pollen (pollination effect). Ovaries were collected 1, 2, 3, 4, 6, 8, and 10 d following pollination (day effect). For fertilization frequency and number of endosperm nuclei, both pollination type and day were treated as fixed effects. F-tests for both fertilization frequency and number of endosperm nuclei used MSresidual as the denominator. The degrees of freedom of the residual mean square were 81 and 27 for the analysis of fertilization and endosperm nuclei, respectively. Means are presented in figs. 2 and 3.

* P < 0.05.
** P < 0.01.
*** P < 0.001.

The evidence based on frequencies of fertilization in self-pollinated and outcrossed flowers clearly supports the presence of a prezygotic mechanism of self-sterility. Fertilization occurred less often in self-pollinated flowers than in outcrossed flowers. Furthermore, the mean values of fertilization frequency (self mean = 0.11, outcross mean = 0.35) were similar to mean seed-set proportions (self mean = 0.09, outcross mean = 0.38), which indicates that additional mechanisms are not required to explain the observed patterns of seed set. No previous studies have directly tested for prezygotic mechanisms in C. borealis, although Galen et al. (1985) and Barrett and Helenurm (1987) considered that self-sterility may be the result of partial self-incompatibility. Our result is also consistent with the observation that self-incompatibility is common in the family Liliaceae (Fryxell 1957). In Lilium longiflorum and Lilium martagon, for example, rejection of self-pollen is prezygotic and occurs in the style (Dickinson et al. 1982; Lunqvist 1991). The specific nature of self-incompatibility and the location of pollen rejection in C. borealis is beyond the scope of this study and would require a quantitative analysis of pollen tube growth rates.

We used divisions of the polar nucleus and zygote as the primary indication that fertilization of the egg cell had occurred in C. borealis embryo sacs. In a small number of cases, fertilization was assessed indirectly based on divisions of the polar nucleus. This approach was necessary because it was not always possible to clearly view the egg (or zygote) in all ovules. Although this approach is widely used in studies of reproductive development, it may lead to inaccurate estimates of fertilization rates, particularly if the polar nucleus can divide in the absence of double fertilization. However, for ovules in which both endosperm and zygote were observed, we found that the number of nuclei in the zygote was positively correlated with the number of nuclei in the endosperm (r = 0.65, P > 0.0001). Moreover, there is no previous evidence to indicate that nuclear divisions in the endosperm commonly occur without fertilization in C. borealis. In fact, in previous studies of self-sterility, the reverse was true: some embryos in self-pollinated flowers were found to develop without endosperm.
division (Manasse and Pinney 1991). As a result, it is likely that measures of the frequency of fertilization based on endosperm division provide an accurate minimum estimate of the frequency of fertilization in the ovary.

Two pieces of evidence indicate that postzygotic processes are also acting to reduce seed set upon selﬁng in C. borealis. First, we found differences in the rate of endosperm division between selﬁed and outcrossed seeds. Although we have no data for this trait beyond 10 d after pollination, reduced endosperm development may result in selﬁed seeds that are smaller and more likely to be aborted early in development (Galen et al. 1985). Hence, it seems likely that low rates of endosperm development after fertilization has contributed to self-sterility in C. borealis. Second, full seeds from outcrossed flowers were larger than those from selﬁed flowers. This difference parallels the observed patterns of endosperm development and will also have important consequences for offspring ﬁtness (Stanton 1984).

While differences in endosperm development and seed size between self- and cross-fertilized ovules are manifested postzygotically, we cannot exclude the possibility that they are the indirect effects of prezygotic mechanisms rather than the direct result of postzygotic processes (inbreeding depression or late-acting incompatibility). For example, seed enlargement can occur in the absence of fertilization and therefore may be independent of the genetic quality of the zygote (Brock 1954). In partially self-incompatible plants, the development of selﬁed fruits and their seeds may be slower because they have lower sink strength for maternal resources, as a result of having fewer seeds. These prezygotic differences may persist and negatively affect offspring at several life-history stages (Becerra and Lloyd 1992). Although the pathways through which prezygotic processes may affect seed development are clear, few experimental studies have quantiﬁed the relative importance of prezygotic events on postzygotic fruit and seed development.

Despite the evidence supporting the existence of a postzygotic component to self-sterility, the underlying cause, either late-acting incompatibility or inbreeding depression, is less clear. Seavey and Bawa (1986) suggest several criteria that could be used to distinguish the rejection response of classic incompatibility systems from the expression of deleterious mutations in inbreeding depression. Among those, they suggest that embryo failure occurring at a number of stages could likely be attributed to inbreeding effects, since inbreeding depression is expected to arise owing to rare mutations at a large number of genes. For the same reasons, inbreeding depression should result in a variable degree of self-sterility among genotypes, assuming that individuals exhibit random variation in the number of deleterious alleles. However, uniform failure of zygotes, both in time and among genotypes, may indicate active rejection as expected with physiological self-incompatibility.

The pattern of endosperm division observed in self-fertilized ovules of C. borealis is consistent with an active incompatibility response. The number of divisions rarely exceeded two, and there was no evidence that endosperm in even a portion of the selﬁed ovules continued to divide as one would predict under an inbreeding model. This pattern of ovule development is in accord with studies on Asclepias syriaca (Sparrow and Pearson 1948; Kahn and Morse 1991), Rhododendron (Williams et al. 1984), Chor-isia, Tabebuia (Gibbs and Bianchi 1993), and Pseudowintera (Sage et al. 1999) that demonstrate only minor endosperm divisions in self-fertilized ovules. Additional observations of endosperm development beyond the 10-d interval are necessary to conﬁrm the result for C. borealis.

The wide variation in seed set among clones observed in C. borealis appears consistent with the effects of inbreeding depression. Seed set in selﬁed ﬂowers ranged from 0 to 0.89 among clones, with 15 of 25 producing no seed. Expressed relative to seed set in outcrossed ﬂowers, seed set in selﬁed flowers was continuously distributed and ranged from 1.88 to 0.10 among clones. This result is in accord with many studies of inbreeding depression that demonstrate high inbreeding depression in the early stages of embryo development and signiﬁcant variation in the effects of selﬁng among plants (Husband and Schemske 1995, 1996). However, even these results are not without some ambiguity. First, despite the wide range of values, differences among clones in self-sterility were not statistically detectable, although the lack of a signiﬁcant patch × pollination interaction may be the result of low power, attributable to low number of clones and high variation among ﬂowers within a clone. Second, variation among selﬁed ﬂowers in the proportion of fertilized ovules per ovary (CV = 117.5 ± 11.8 SE) was higher than for seed set (CV = 89.3 ± 11.3 SE), which indicates that variation in seed set may be associated with variation in fertilization rates and the strength of self-incompatibility. Because of these uncertainties in the interpretation of the seed set data, we feel it is likely but perhaps premature to conclude that inbreeding depression is an important factor causing postzygotic self-sterility in C. borealis. A crossing experiment involving plants of different degrees of relatedness would provide a more deﬁnitive test of the role of inbreeding depression in postzygotic self-sterility (Seavey and Carter 1994).

The presence of both post- and prezygotic mechanisms of self-sterility in plants is rarely reported in the literature, perhaps because both are rarely examined in the same plants. Most studies of self-compatible species focus on inbreeding depression (Husband and Schemske 1996), despite the fact that prezygotic factors such as cryptic and partial self-incompatibility may also inﬂuence seed production and offspring ﬁtness. Microscopic studies of ovule development and fertilization, infrequently used in studies of inbreeding depression, are more likely to encounter both forms of sterility. For example, Manasse and Pinney (1991) found lower fertilization and higher abortion in selﬁed ﬂowers of Crinum erubescens, although they attributed most of the self-sterility to postzygotic effects. Similarly, studies of Delphinium (Waser and Price 1993, 1994) have revealed both prezygotic as well as postzygotic mechanisms of sterility in inbred matings. Despite the rarity of such a result, the presence of both post- and prezygotic effects on seed production may not be surprising. A species that is partially incompatible would, depending on the history of inbreeding, be expected to maintain relatively high genetic load and hence exhibit postzygotic patterns of self-sterility (Levin 1984; Levin and Bulinska-Radomska 1988). Alternatively, many species that are self-compatible may produce fewer seeds under selﬁng and thus are partly self-sterile. In C. borealis, prezygotic effects may be the dominant mechanism underlying partial self-sterility, but postzygotic effects exist and may con-
tribute to the wide variation among individuals. A better understanding of the magnitude of pre- and postzygotic mechanisms and their interactions may provide further insight into the basis and evolution of partial sterility in other plants, an advance that is likely to arise only by direct examination of pollination, double fertilization, and embryo development.

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