American Journal of Botany 93(2): 254–262. 2006.

SELF-STERILITY IN *IPOMOPSIS AGGREGATA* (POLEMONIACEAE) IS DUE TO PREZYGOTIC OVULE DEGENERATION[1]

TAMMY L. SAGE,[2,4] MARY V. PRICE,[3] AND NICKOLAS M. WASER[3]

[2]Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2; [3]Department of Biology, University of California, Riverside, California 92521 USA; and Rocky Mountain Biological Laboratory, Crested Butte, Colorado 81224 USA

Based on previous studies, extreme (>99%) self-sterility in scarlet gilia (*Ipomopsis aggregata*) appears to be involved in late-acting ovarian self-incompatibility (OSI). Here, we confirm this suggestion by comparing structural events that follow from cross- vs. self-pollinations of *I. aggregata*. Growth of cross- and self-pollen tubes in the style at 11 h and growth in the ovary at 24 h was equivalent. Nonetheless, by 24 h, cross-pollen effected a significantly higher percentage of both ovule penetration and fertilization. Ovules in self-pollinated flowers showed pronounced changes, including an absence of embryo sac expansion and reduced starch in the integument, by 11 h post-pollination, well before pollen tube entry into the ovary. In addition, the integumentary tapetum and adjacent 1–3 cell layers exhibited abnormal cell division, pronounced deposition of thick, pectin-rich cell walls, and cellular collapse. Ovules and embryo sacs from cross-pollinated flowers rarely showed such features. Developmental changes in ovules from self-pollinated flowers eventually resulted in integument and embryo sac collapse, a process not observed in ovules of unpollinated flowers. We suggest that OSI involves long-distance signaling between self-pollen or self-pollen tubes and carpel tissue that reduces availability of receptive ovules for fertilization before pollen tubes arrive in the ovary.

Key words: histology; long-distance signaling; ovarian self-incompatibility (OSI); ovular collapse; Polemoniaceae; Rocky Mountain Biological Laboratory; self-incompatibility.

Self-incompatibility (SI) is a genetically controlled process in angiosperms that results in the recognition and rejection of self- or self-related pollen and pollen tubes (de Nettancourt, 1977, 1997, 2001). In some cases, rejection occurs at the stigma through instantaneous post-pollination reactions that inhibit or retard pollen hydration and germination or growth of pollen tubes soon after pollen germination (Hecht, 1964; Knox, 1973; Owens, 1981; Heslop-Harrison, 1982; Franklin-Tong et al., 1994; Pontieri and Sage, 1999; Sage et al., 2001; Bernhardt et al., 2003). Depending on the species, such stigmatic SI may be sporophytic (SSI; incompatibility type determined by diploid S-genotype of the sporophytic pollen parent) or gametophytic (GSI; incompatibility determined by the haploid pollen genotype; de Nettancourt, 1977, 1997, 2001). In other cases, rejection of self-pollen occurs in the style through reactions that inhibit growth of self-pollen tubes (Crane and Lawrence, 1929; Crane and Brown, 1937; Herrero and Dickinson, 1980). To date, all stylar SI is documented to be gametophytic (de Nettancourt, 1977, 1997, 2001).

Self-rejection in some species may not affect self-pollen germination and tube growth even though selfing is followed by sterility (Seavey and Bawa, 1986; Sage et al., 1994). In one version of such late-acting ovarian SI (OSI), self-pollen tubes enter ovules but do not penetrate embryo sacs (Kenrick et al., 1986). In other cases, self-pollen tubes deposit sperm into the embryo sac but double fertilization is not complete (Cope, 1962). In still other instances, double fertilization occurs but zygotes arising following self-pollination never divide (Sears, 1937; Sparrow and Pearson, 1948; Williams et al., 1984; Sage and Williams, 1991; Gibbs and Bianchi, 1993; 1999; Gibbs et al., 1999; Lewis and Gibbs, 1999; Sage and Sampson, 2003; Bittencourt and Semir, 2005). Because inbreeding depression is expected to cause embryo failure at a variety of developmental stages, the uniform failure of zygotes arising from self-pollination at an initial stage prior to cell division differentiates this last form of OSI (postzygotic OSI) from inbreeding depression (Charlesworth, 1985; Seavey and Bawa, 1986). Furthermore, the high degree of self-sterility of some OSI species also precludes inbreeding depression as an explanation of developmental failure (see Appendix I of Waser and Price, 1991). Once considered to be an anomalous phenomonon (de Nettancourt, 1977), OSI is now known to be widely distributed amongst flowering plants (Seavey and Bawa, 1986; Sage et al., 1994; Gibbs and Bianchi, 1999; Gibbs et al., 1999; Bittencourt et al., 2003; Pound et al., 2003; Sage and Sampson, 2003; Bittencourt and Semir, 2005) and hence, to play an important role, as do other forms of SI, in reducing inbreeding and its harmful effects (Charlesworth and Charlesworth, 1987; Barrett, 1988; de Nettancourt, 1997; Waser and Williams, 2001). A limited number of studies indicate that OSI is under genetic control (Cope, 1962; Lipow and Wyatt, 2000; LaDoux, 2004).

Self-sterility in the montane perennial *Ipomopsis aggregata* (Pursh) V. Grant (Polemoniaceae) is extremely high, with greater than 99% of ovules failing to develop following self-pollination (Waser and Price, 1991). Self-sterility in this species is not due to SI reactions that prevent self-pollen from germinating on the stigma, growing in the style, or penetrating ovules (Waser and Price, 1991). Rather, self-sterility appears to be due to late-acting OSI because ovules that are penetrated by self-pollen tubes appear to degenerate as indicated by reduced size and appearance of a milky substance interpreted to be callose (Waser and Price, 1991). Since self-pollen tubes of *I. aggregata* enter ovules, self-sterility may be postzygotic, although this has not been demonstrated.

We compared ovule and seed development following cross-, self-, and no pollination in *I. aggregata* to assess (1) whether SI

[1] Manuscript received 28 July 2005; revision accepted 1 November 2005.

The authors thank Peter Drobac for technical assistance. Research was funded by a Natural Sciences and Engineering Research Council of Canada grant to T.L.S.

[4]Author for correspondence (tsage@botany.utoronto.ca)

is pre- or postzygotic; (2) temporal aspects of ovule and seed degeneration following self-pollination; and (3) the anatomical and histochemical features of ovule and seed degeneration. Our findings confirm OSI in *I. aggregata* and indicate that the phenomenon is prezygotic, apparently involving a modification of long-distance signaling between pollen or pollen tubes and stigmatic or stylar tissue on the one hand and ovarian tissue on the other.

MATERIALS AND METHODS

Study organism—Scarlet gilia, *Ipomopsis aggregata*, is a monocarpic herbaceous perennial that is widespread in montane areas of western North America (Grant and Wilken, 1986). Plants may grow for 2–7 yr as a vegetative rosette prior to producing up to several hundred nectar-producing red, tubular flowers. Populations of *I. aggregata* used for this experiment were located at or near the Rocky Mountain Biological Laboratory (RMBL) in western Colorado. The predominant pollinators of *I. aggregata* growing at RMBL are hummingbirds, although flowers are also visited by insects from several orders, some of them effective pollinators, including bumble bees, solitary bees, syrphid and muscoid flies, and hawkmoths and butterflies (Price et al., 2005).

Pollination treatments—Two separate sets of controlled pollinations were conducted to determine whether there were differences in the time course and nature of ovule and seed development following cross- and self-pollination. In late June 1998, we potted five adult plants from each of two sites separated by ca. 10 m within a population at the RMBL. These plants were potted before flowering and were maintained in a greenhouse at the RMBL. Similarly, in late June 1999, we potted six adult plants from each of two sites separated by ca. 10 m within a population 4 km south of the RMBL. These plants were taken to the University of Toronto where they were maintained in a glasshouse at 25°C until flowering was complete. In both experiments, flowers were emasculated in the bud stage so that stigmas were free of any pollen when they became receptive several days later, as indicated by reflexed stigma lobes. On each plant, controlled hand pollinations were conducted by applying either pure cross- or self-pollen on all three stigmatic lobes of separate flowers, alternating the treatments from one flower to the next. All cross-pollinations were between plants from the two sites in the source population separated by ca. 10 m. Pollinated carpels were harvested at 11 h, 24 h, 48 h, and 120 h post-pollination. Unpollinated carpels were also harvested at the same times. Time zero for emasculated, unpollinated carpels was determined as the time when stigmas became receptive. Harvested pollinated and unpollinated carpels were fixed in either formalin–acetic acid–alcohol (FAA; 1998 experiment) or in glutaraldehyde (1999 experiment) as described by Sage and Williams (1995), dehydrated in ethanol, embedded in LR White resin (Electron Microscopy Supplies, Fort Washington, Pennsylvania, USA), and serially sectioned at 1.5 μm with a diamond histoknife (Delaware Diamond Knives, Wilmington, Delaware, USA). Carpel tissue fixed in glutaraldehyde only was used for quantitative observations because of the absence of cell distortion.

Quantitative analysis of pollen tube growth—Stigmas, styles, and ovaries from serially sectioned carpels were examined to assess pollen tube growth following cross- vs. self-pollination to insure that OSI was indeed functioning as previously described (Waser and Price, 1991). To determine whether or not there were differences between cross- vs. self-pollen tube growth within the carpel at 11 and 24 h post-pollination, the number of germinated pollen grains on the stigma, pollen tubes at the base of the style and mid-ovary, and micropyles penetrated by pollen tubes were quantified for a subset of sectioned carpels ($N = 1$ carpel per plant per pollination treatment per sample time; five plants). These times were selected because Waser and Price (1991) noted that ovule degeneration had been initiated by 24 h. Sectioned tissue was used to quantify pollen tube growth because *I. aggregata* does not deposit callose in the pollen tube walls within the style (Waser and Price, 1991; T. Sage, personal observations), thereby preventing the use of squashes of softened pollinated carpels to detect callose in pollen tube walls with fluorescence microscopy (Martin, 1959). For each carpel, the number of cross- or self- pollen tubes at the base of the style or mid-ovary divided by the number of germinated pollen grains per stigma was multiplied by 100. The number of penetrated micropyles was divided by the number of ovules within the carpel and multiplied by 100. The values for cross- vs. self-pollen tubes at the base of the style were contrasted with a Student's t test as were those for cross- vs. self- pollen tubes at mid-ovary and micropyles penetrated by cross- vs. self-pollen tubes. All statistical analyses were performed using Sigma Stat (version 2.03; SPSS, Inc., Chicago, Illinois, USA).

Ovule, seed, and embryo sac development following cross-, self-, and no pollination—Anatomical and histochemical features of ovule, seed, and embryo sac development following each pollination treatment were assessed after staining the majority of sections with periodic acid-Schiff's reagent (PAS; O'Brien and McCully, 1981) and counterstaining with toluidine blue (O'Brien and McCully, 1981). To characterize the nature of the milky ovular occlusion reported to be present in the ovule micropyle following self- vs. cross-pollination (Waser and Price, 1991), a subset of sections ($N = 3$ carpels per plant per pollination treatment; five plants) was stained with either aniline blue to detect callose (Martin, 1959), phloroglucinol to detect lignin (O'Brien and McCully, 1981), or ruthenium red to detect pectins (O'Brien and McCully, 1981).

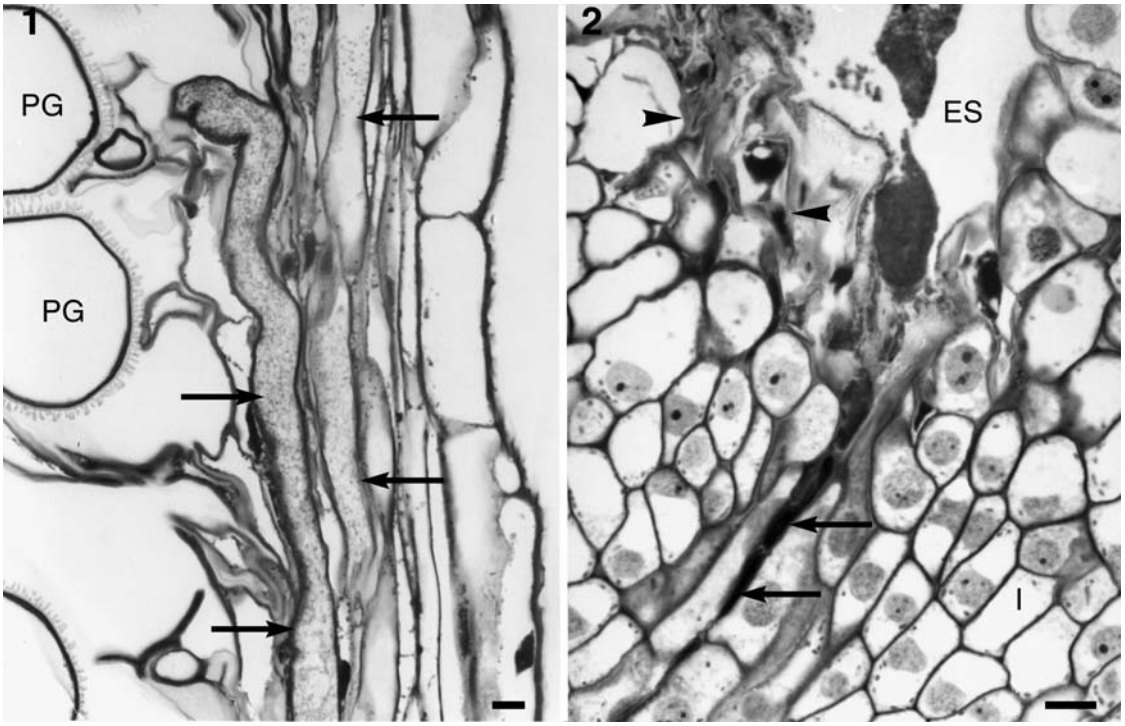
Microscopic examination of stained serial ovary sections to assess general structural and histochemical features of ovule and seed development following cross- vs. self-pollination ($N = 14$ carpels per plant per pollination treatment per harvest time; 11 plants) revealed a qualitative difference in the amount of starch in the integument of ovules from cross- vs. self-pollinated flowers at 11 h. Furthermore, although small plastids were present in the integument of unpollinated flowers, starch grains were absent. Therefore, the amount of integument area occupied by starch at 11 h was quantified with imaging software (Image J; National Institutes of Health, Bethesda, Maryland, USA) at the median longitudinal section of a subset of sectioned carpels fixed in glutaraldehyde ($N = 3$ ovules per ovary; one ovary per plant per pollination treatment; four plants). Initially, a one-way ANOVA was used to determine if there were any significant differences in integumentary starch between ovaries developing on different plants within a pollination treatment. No differences were observed. Therefore, all data within treatments were pooled. Statistical analyses were then performed to determine whether there were differences in the amount of starch in the integument at 11 h between pollination treatments using an ANOVA on ranks with multiple comparison procedures (Dunn's or Tukey's).

Serially sectioned carpels were also used to quantitatively determine the timing of ovule and seed collapse following cross- vs. self-pollination that was reported by Waser and Price (1991). The median longitudinal section of ovules and seeds and the enclosed embryo sacs from cross-, self-, and unpollinated flowers of glutaraldehyde-fixed tissue at 11 h, 24 h, and 120 h ($N = 6$ ovules–seeds–embryo sacs per ovary; one ovary per plant per pollination treatment per sample time; five plants) were used to quantify the area of ovules, seeds, and embryo sacs with the 10× objective of a Zeiss Axioplan microscope (Carl Zeiss, Göttingen, Germany) equipped with an image analysis system (Northern Exposure; Empix Imaging, Mississauga, Ontario, Canada). Initially, a one-way ANOVA was used to determine whether there were any significant differences in ovule, seed, and embryo sac area between ovules, seeds, and embryo sacs from different ovaries developing on different plants within a pollination treatment at each harvest time. No differences were observed. Therefore, all data within treatments for each harvest time were pooled. Statistical analyses were then performed to determine whether there were differences between ovule, seed, and embryo sac area between pollination treatments at each harvest time using one-way ANOVA on ranks with multiple comparison procedures (Dunn's or Tukey's). Serial sections from unpollinated carpels used for quantification of ovule, seed, and embryo sac area as well as starch occupied on an area basis were examined to insure that pollination had not occurred.

Quantification of double fertilization—Serial sections of ovaries were used to assess the percentage of ovules with double fertilization ($N = 3$–4 ovaries per plant; three plants) at 24 and 48 h after cross- vs. self-pollination. The mean percentage of double fertilization following cross- vs. self-pollination was then compared using a Mann-Whitney rank sum test. Double fertilization was indicated by the presence of: (1) pollen tube penetration of the embryo sac; (2) a resting zygote or embryo; and (3) one or more endosperm nuclei.

RESULTS

Pollen tube growth following cross- vs. self-pollination—Pollen tubes initially grew intercellularly amongst ground cells adjacent to stigmatic epidermal cells (Fig. 1) and then to the



Figs. 1–2. Intercellular growth of self-pollen tubes of *Ipomopsis aggregata*. **1.** In substigmatic tissue at 11 h. **2.** In micropyle at 24 h post-pollination. Arrows mark pollen tubes. Arrowheads denote collapsed integumentary cells. Bars = 10 μm. Abbreviations: ES, embryo sac; I, integument; PG, pollen grain.

base of the style by 11 h following cross- and self-pollination. Pollen tubes contained continuous cytoplasm along their entire length from the stigma to the ovary. No significant differences were observed between the mean percentage of pollen tubes from germinated cross- vs. self-pollen at the base of the style at 11 h (cross = 72.0 ± 7.9%; self = 64.6 ± 10.9% [mean ± 95% confidence interval]; df = 8, $t = 1.518$, $P = 0.167$) or at the mid-ovary at 24 h (cross = 25.8 ± 9.8%; self = 30.6 ± 10.9%; df = 8; $t = -0.892$; $P = 0.399$). While both cross- and self-pollen tubes entered micropyles by 24 h (Fig. 2), significant differences were observed in the percentage of micropyles penetrated (cross = 29.2 ± 8.5%; self = 7.0 ± 6.2%; df = 8; $t = -5.833$; $P \leq 0.001$).

Ovule, seed, and embryo sac development—Striking developmental differences between ovules from self-, cross-, and unpollinated flowers were apparent at 11 h post-pollination, well before ovary and hence micropyle penetration by pollen tubes. At this time, the mean area of the median section of ovules and embryo sacs was significantly less following application of self- vs. cross-pollen or no pollen (Fig. 3). As well, although starch grains were present in the integument following pollination only, the percentage area of the median section of integument occupied by starch grains was significantly less following self- vs. cross-pollination (Figs. 4, 5; outcross = 19.3 ± 2.4%; self = 5.2 ± 0.9%; unpollinated = 0 ± 0%; df = 2; $H = 32.347$; $P \leq 0.001$). The innermost layer of the integument adjacent the embryo sac, hereafter referred to as the integumentary tapetum (Kapil and Tiwari, 1978), was characterized by hypertrophied cells with localized regions of cell divisions that were periclinal to the surface of the embryo sac at 11 h following self-pollination (Fig. 6). These localized cell divisions were in some instances organized into proembryos (Fig. 7; adventitious embryony; Koltunow, 1993). Cellular collapse of integumentary tapetum cells was common at 11 h following self-pollination (Figs. 6–9). In addition, the integumentary tapetum and 1–3 adjacent layers of integumentary cells developed thick cell walls (Figs. 6–9) that were PAS- and ruthenium red-positive but phloroglucinol- and aniline blue-negative. As observed for the integumentary tapetum, adjacent integumentary cells also exhibited cellular collapse (Figs. 7, 8). The cellular features apparent in ovules at 11 h following self-pollination became more pronounced at 24 and 48 h. By 120 h, the embryo sac and cells comprising the integument collapsed (Figs. 3, 10). Histochemical studies indicated an absence of micropyle exudate in all ovules examined from self-pollinated carpels.

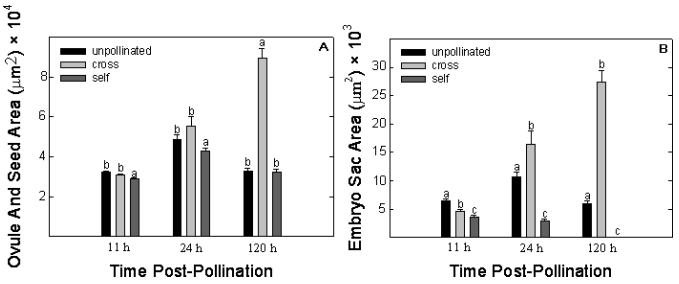
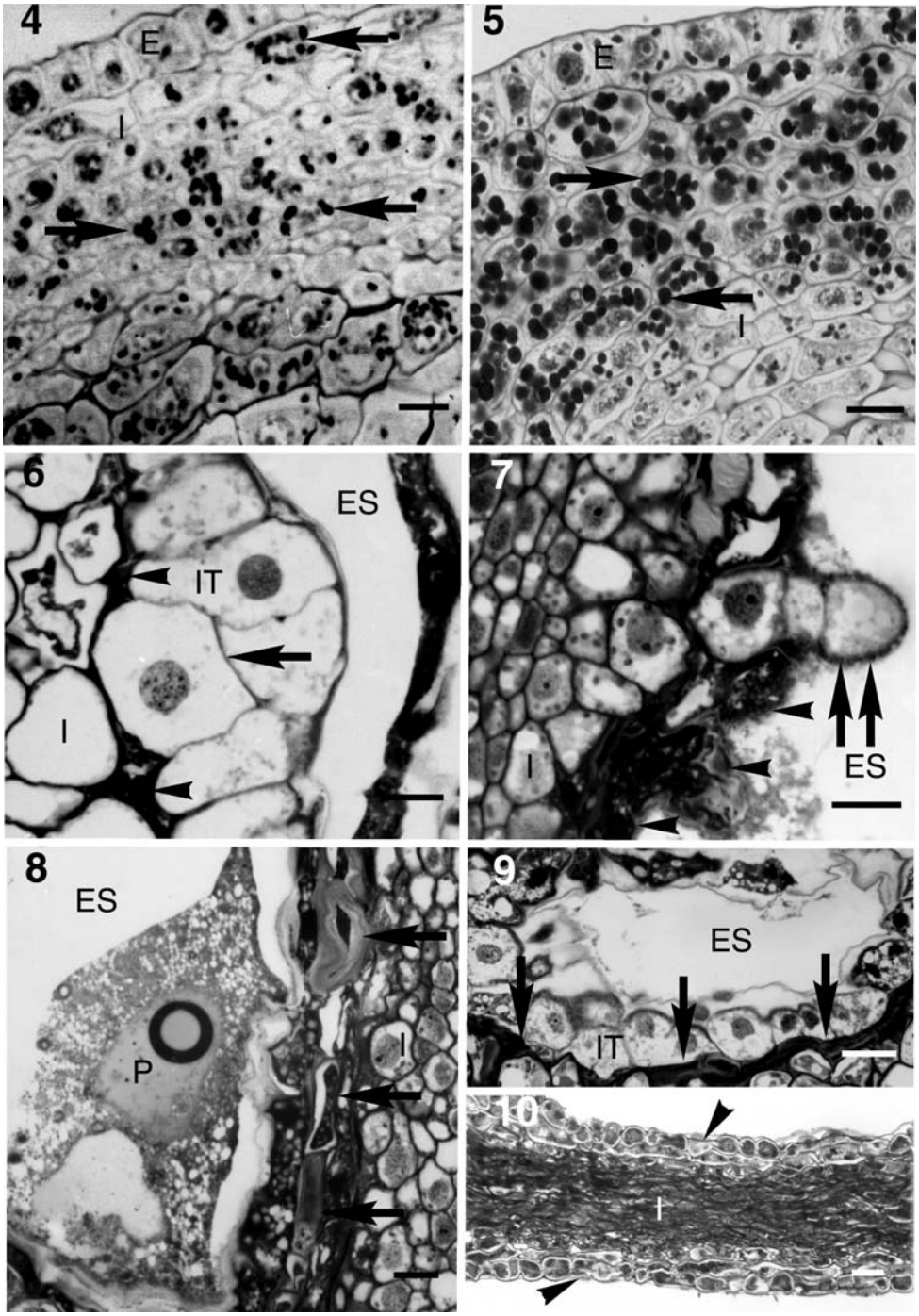
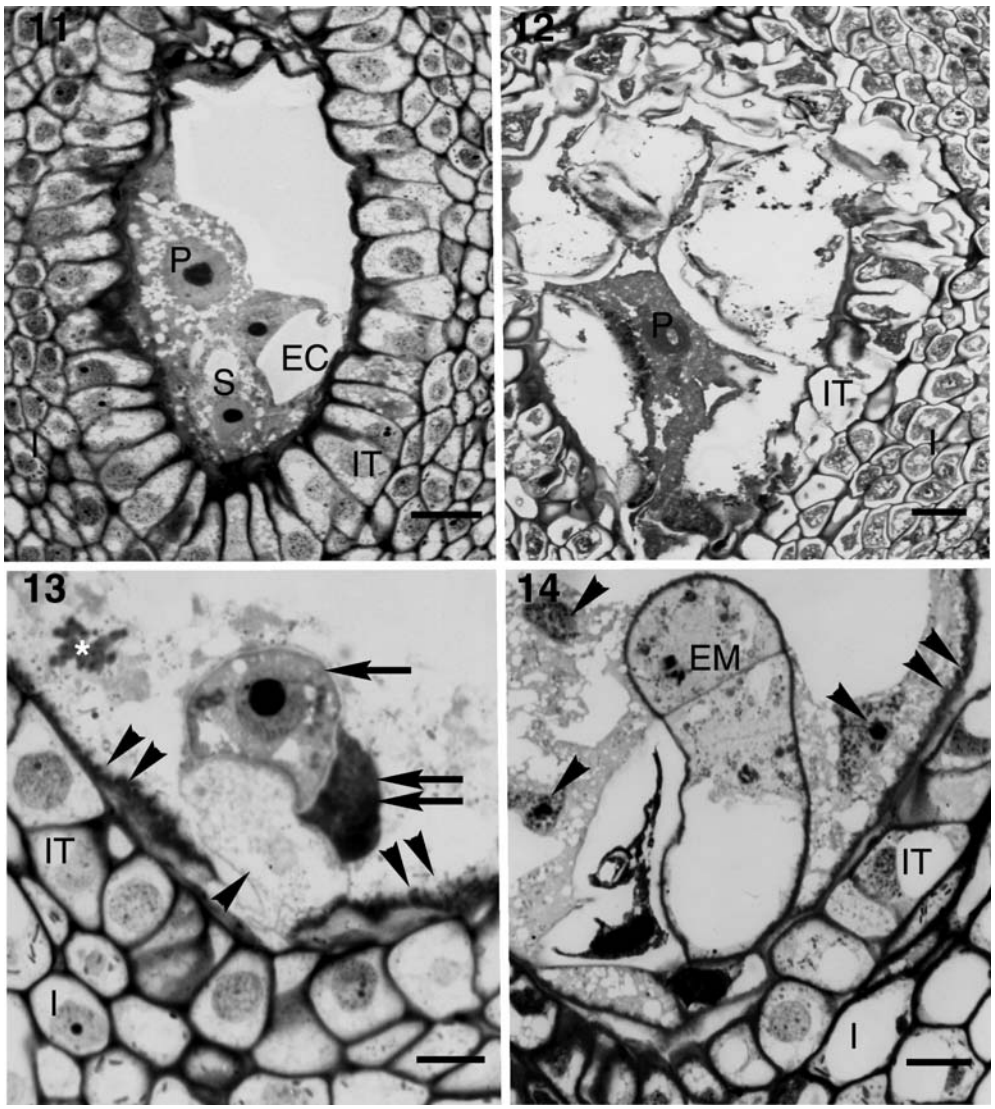


Fig. 3. Mean area of (A) ovule and seed and (B) embryo sac following cross-, self- and no pollination of *Ipomopsis aggregata*. Values within a treatment time with different letters are significantly different from one another at $P \leq 0.05$. Error bars represent 95% confidence intervals.



Figs. 4–10. Ovule development following cross- and self-pollination of *Ipomopsis aggregata*. Figs. 4–9, 11 h post-pollination. Fig. 10, 120 h post-pollination. **4, 5.** Starch grains (arrows) within self- (4) vs. cross- (5) integument. **6.** Periclinal division of integumentary tapetum cells following self-pollination (arrow). Arrowheads denote pectin-rich wall of integument cells. **7.** Apomictic embryo (double arrows) arising from integumentary tissue following self-pollination. Note collapsed integumentary cells (arrowheads). **8.** Arrows denote thickened pectin-rich cell walls of collapsed integumentary cells following self-pollination. **9.** Pectin-rich boundary (arrows) of integument adjacent embryo sac following self-pollination. **10.** Collapsed ovule following self-pollination. Arrowheads denote epidermis. Bars = 10 μm. *Abbreviations*: E, epidermis; ES, embryo sac; I, integument; IT, integumentary tapetum; P, polar nucleus.

Ovules from unpollinated and cross-pollinated flowers were anatomically distinct from ovules of self-pollinated flowers at all sample times. At 11 h following cross-pollen application and no pollination, divisions in the integumentary tapetum periclinal to the embryo sac were absent as were adventitious embryos. Rather, the integumentary tapetum was organized into a discrete single layer of cells arising from divisions anticlinal to the embryo sac (Fig. 11). The cellular collapse and deposition of thickened PAS- and ruthenium red-positive cell walls adjacent to the integumentary tapetum and subsequent



Figs. 11–14. Ovule and seed development following cross-pollination (Figs. 11, 13, 14) and no pollination (Fig. 12) of *Ipomopsis aggregata*. **11.** Embryo sac with egg cell, one of two synergids, and polar nucleus in pollinated flower at 11 h post-stigma receptivity. Note orderly array of integumentary tapetal cells. **12.** Embryo sac with polar nucleus in unpollinated flower at 120 h after stigma receptivity. **13.** Resting zygote (single arrow), penetrated synergid (double arrows), and unpenetrated synergid (single arrowhead) at 24 h post-pollination. Double arrowheads denote transfer cell walls of embryo sac adjacent integumentary tapetum. An asterisk marks endosperm nucleus. **14.** Four-celled embryo at 120 h post-pollination. Arrowheads mark endosperm nuclei. Double arrowheads denote transfer cell walls of embryo sac adjacent integumentary tapetum. Figs. 11, 13, 14, bars = 10 μm. Fig. 12, bar = 20 μm. Abbreviations: EC, egg cell; EM, embryo; I, integument; IT, integumentary tapetum; P, polar nucleus; S, synergid.

integument-embryo sac collapse that was observed following self-pollination were not present in the absence of pollination (Figs. 3, 12), even though the embryo sac and integumentary cells of ovules from unpollinated carpels appeared senescent by 120 h (Fig. 12). The cellular features of ovules associated with self-pollination were only observed following cross-pollination in six seeds from one ovary from one plant at 24 h, two seeds from one ovary from one plant at 48 h, and four seeds from one ovary from a second plant at 120 h. Histochemical studies indicated an absence of micropyle exudate in all ovules examined from unpollinated and cross-pollinated carpels.

Double fertilization was evident by 24 h in all ovules penetrated by cross-pollen tubes, as indicated by entry of pollen tubes into a synergid and by the presence of resting zygotes and four endosperm nuclei in an embryo sac with well-defined transfer cell walls (Fig. 13). In contrast, double fertilization had not occurred by 24 h in any of the ovules penetrated by self-pollen. By 48 h, seeds from cross-pollinated flowers contained either a resting zygote or a two-celled embryo and 16 endosperm nuclei. Seeds from self-pollinated flowers contained only a resting zygote and four endosperm nuclei at 48 h. The percentage of double fertilization was greater following cross- vs. self-pollination at 48 h (cross = 83.1 ± 7.9%; self = 1.3 ± 2.8%; $T = 187$; $P \leq 0.001$). A four-celled embryo (Fig. 14) with greater than 32 endosperm nuclei was apparent in all fertilized seeds by 120 h following cross-pollination only. Ovule, seed, and embryo sac area at the median longitudinal section increased from 11 to 120 h following cross-pollination (Fig. 3).

DISCUSSION

We have demonstrated that the recognition of self-pollen in *I. aggregata* flowers is prezygotic and is associated with abnormal development of the integument and embryo sac of ovules. This abnormal development begins before self-pollen tubes penetrate the ovary and results ultimately in the collapse of ovules, even though self-pollen tubes may subsequently enter the micropyle, and in rare cases, effect double fertilization. We begin by discussing the implications of these findings for the mechanisms of self-recognition and rejection in *I. aggregata*. Because OSI in this species results in prezygotic initiation of events that lead to abortion of ovules, we next address the functional consequences for reproduction. We conclude by comparing our results with what is known about SI in other genera within the Polemoniaceae.

Mechanism of OSI in I. aggregate—The observations reported here indicate that the process of self-recognition and rejection in *I. aggregata* entails long-distance signaling between self-pollen or self-pollen tubes and stigmatic or stylar tissue on the one hand and ovarian tissues on the other, that results in the modification of one or more post-pollination stimulatory functions of pollen or pollen tubes on ovule tissue development. This signaling, and the reactions to it, occur at least by 11 h following pollination and are distinct from those that occur in the absence of pollination and after cross-pollination because the histological and anatomical results are so clearly different. Whereas cross-pollen or cross-pollen tubes elicit significant starch storage in the integument, induce an orderly development of the integumentary tapetum and adjacent cell layers before pollen tubes enter the ovary, the presence of self-pollen or self-pollen tubes instead elicits little starch storage, disorderly development of the integumentary tapetum and adjacent cells, and a reduction in embryo sac area in comparison to both cross-pollinated and unpollinated flowers. In the absence of pollination, no starch storage occurred even though integument development was not significantly different from that following cross-pollination.

There is good evidence from other flowering plants for long-distance signaling in which the presence of compatible pollen or pollen tubes influences ovule development, embryo sac viability, starch metabolism, and transmitting tissue secretion in ovarian tissue prior to entry by pollen tubes (Pimienta and Polito, 1982, 1983; Herrero and Gascon, 1987; Herrero and Arbeloa, 1989; O'Neill, 1997; Pontieri and Sage, 1999; Koehl, 2002). And, although the distances of signaling are less than stigma-to-ovule, a form of pollen-to-carpel signaling has been documented in *I. aggregata* whereby pollen application results in closure of stigma lobes (Waser and Fugate, 1986).

Ipomopsis aggregata represents the first eudicot OSI species and second flowering plant in which SI reactions result not in altered pollen tube development, but instead in altered ovule development prior to entry of ovaries by pollen tubes. Self-pollination in the monocotyledonous OSI species *Narcissus triandrus* also resulted in failed ovule development that was initiated well before pollen tubes entered the ovary (Sage et al., 1999). These results are important because they broaden the taxonomic range of species reported to exhibit OSI that is prezygotic in its action. In contrast to the negative prezygotic affect of self-pollen on ovule development reported here for *I. aggregata* and elsewhere for *N. triandrus* (Sage et al., 1999), a study on SI in the basal angiosperm lizard's-tail (*Saururus cernuus*) indicated that even in the presence of SI mechanisms that operate to prevent self-pollen hydration and germination at the stigma, long-distance signaling from self-incompatible pollen still stimulates ovule development, a phenomenon that also occurs following cross-compatible pollinations but not in the absence of pollination (Pontieri and Sage, 1999). Our results for *I. aggregata* reinforce the view proposed by Sage et al. (1999) that such OSI phenomena be included within a broadened functional view of SI that recognizes prezygotic maternal recognition of self as the fundamental feature of all incompatibility systems, regardless of the underlying molecular and physiological mechanisms involved.

Functional consequences of self-pollination on reproduction in I. aggregate—Our data show that self-sterility following application of only self-pollen in *I. aggregata* was due to an absence of fertilization. This absence in fertilization occurred as a result of a reduction in the number of ovules penetrated by self-pollen tubes even though the number of pollen tubes present in the ovary was not significantly different between the two pollination treatments. We posit that developmental changes in ovules that occurred following self-pollination had a negative impact on the ability of ovules to attract pollen tubes. A similar reduction in self-fertilization due to the negative influence of self-pollen on ovule development has also been reported for *Narcissus triandrus* (Sage et al., 1999). Significantly, self-pollination in *N. triandrus* resulted in a low level of self-seed set (Sage et al., 1999). Self-seed set occurred because ovule development in *N. triandrus* was asynchronous, thereby placing some ovules outside of a developmental window where they could not be negatively influenced by self-pollen or self-pollen tubes (Sage et al., 1999). We posit that the very low rate of self-fertilization observed in the present study and low levels of seed set following self-pollination in *I. aggregata* (Waser and Price, 1991) may also be due to physiological asynchrony in ovule development, which cannot be observed at the anatomical level. Staggered ovule development may provide reproductive assurance (Sage et al., 1999).

Stigmas in natural populations may experience mixed cross- and self-pollen loads and the role of mixed cross- and self-pollen loads on fecundity has been a long-standing topic of interest (Galen et al., 1989). A common observation in OSI species is that the application of self-pollen either before or mixed with cross-pollen results in reduced seed set (Cope, 1962; Dulberger, 1964; Waser and Price, 1991; Lloyd and Wells, 1992; Broyles and Wyatt, 1993; Barrett et al., 1996b). This reduction has been referred to as "ovule usurpation" by Waser and Price (1991) and "ovule-discounting" by Barrett et al. (1996b). In the case of *I. aggregata*, Waser and Price (1991) reported a substantial decrease in seed set when self-pollen was applied to the stigma (separate lobes) either at the same time as cross-pollen or 9 h before. Notably, only a small percentage of the reduction in seed set could be accounted for by a decline in cross-pollen deposition or adherence of cross-pollen to stigmas, suggesting that additional mechanisms result in lower seed set with mixtures of self- and cross-pollen. The developmental consequences following application of only self-pollen to stigmas of *I. aggregata*, ovule collapse during the first 120 h following pollination, may serve as a valuable indicator to confirm whether or not prezygotic reduction in ovule availability also occurs in the presence of pollen loads containing both cross- and self-pollen on the stigma in natural populations.

Given the common presence of resource limitations on reproduction in flowering plants, the wastage of ovules in OSI species following self-pollination or pollination with mixed pollen loads appears to be maladaptive because of the loss in reproductive potential that it may entail (Seavey and Bawa, 1986). However, it has been posited that by minimizing investment of maternal resources in selfed-seed during a reproductive episode, plants with OSI may ultimately save resources, compared with those that invest in selfed-seeds that make little contribution to fitness because of inbreeding depression (Sage et al., 1999). It is therefore significant that the first histological and developmental signs of prezygotic reduction in ovule availability following self-pollination in *I. aggregata* include a reduction in integumentary starch in addition to collapse of integumentary cells adjacent the embryo sac and deposition of thick pectin-rich integumentary cell walls that results in a boundary between the integument (sporophyte) and embryo sac (gametophyte). Notably, these microscopic changes occur prior to a metabolically costly two- to three-fold increase in embryo sac area that was observed between 11 h and 24 h following cross- and no pollination. Integumentary carbohydrates are essential for successful ovule and seed growth (Pimienta and Polito, 1983; Sage and Webster, 1990; Zinselmeier et al., 1999). In addition, the integumentary tapetum and adjacent cells in angiosperms function in the regulation of assimilates from the sporophyte to the gametophyte, two generations that do not share a common symplast (Kapil and Tiwari, 1978; Cheng et al., 1996; Wobus and Weber, 1999; Weschke et al., 2003). Along these lines, we report the presence of transfer cell walls in the embryo sac adjacent the integumentary tapetum of *I. aggregata* ovules at 24 h following cross-fertilization only. Transfer cell walls are common at the sporophyte-to-gametophyte junction in all land plants, where they are posited to be the site of metabolite transfer between the two symplastically isolated generations, and they can be induced to develop in the presence of sugars (Offler et al., 2002). Cellular hypertrophy, hyperplasia, and collapse of the integument are also correlated with seed abortion in other species, whereby the deviant integument development is posited to form a physical barrier preventing the flow of assimilates to the embryo sac (Cooper and Brink, 1945; Satina et al., 1950; Miller and Chourey, 1992). Embryo abortion in almond is accompanied by callose deposition around the embryo sac and a decrease in metabolite supply to the embryo sac (Pimienta and Polito, 1982).

Self-incompatibility in the Polemoniaceae—Self-incompatibility is widespread in the Polemoniaceae (Grant and Grant, 1965). A unique feature of the taxon is the presence of multiple types of SI with apparent differences in spatial and genetic control. Self-incompatibility in *Polemonium viscosum*, *Phlox drummondii*, and *Linanthus parviflorus* results in failure of germination of bicellular pollen at a dry stigmatic surface (Levin, 1975, 1993; Galen et al., 1989; Goodwillie, 1997). Self-incompatibility in *P. drummondii* is likely under gametophytic control (Levin, 1975, 1993) whereas SI in *L. parviflorus* is reportedly under sporophytic control (Goodwillie, 1997). In addition, a multi-locus SI system is present in *Ipomopsis tenuifolia* and has been classified as late-acting due to the failure of self-pollen tube growth in the ovary (LaDoux, 2004). On the one hand, different types of SI may have evolved on multiple independent occasions in the taxon (Barrett et al., 1996a). Alternatively, Goodwillie (1997) has proposed that SI in the Polemoniaceae may be a complex genetic system whereby mechanisms of recognition and rejection under sporophytic control are acting in tandem with an ancestral gametophytic system.

Results from the present study on OSI in *I. aggregata* add another layer of complexity to the SI phenomenon in the Polemoniaceae because self-recognition and rejection does not result in failure of pollen germination at the stigma as observed in *Polemonium viscosum*, *Phlox drummondii*, and *L. parviflorus*. Rather, self-recognition results in failure of ovule development in the absence of differential cross- and self-pollen tube growth. Barrett (1988) has proposed that within the angiosperms, ancestral SI systems may be some form of OSI. Does OSI in *I. aggregata* represent yet another type of SI to have evolved within the Polemoniaceae? Or, does it represent an ancestral condition within the taxon? If OSI is ancestral in the Polemoniaceae, would OSI still be acting in tandem with stigmatic SI? Interestingly, application of self-pollen to stigmas 24 h prior to cross-pollen application resulted in a reduction of cross-seed set in *Polemonium viscosum* (Galen et al., 1989). Only a portion of the decline in seed set in *P. viscosum* could be attributed to interference from self-pollen on cross-pollen performance at the stigma (Galen et al., 1989). Similar studies conducted on *I. aggregata* yielded the same results, and Waser and Price (1991) attributed the remaining reduction in cross-seed set to an additional post-pollination effect. Future studies examining the influence of self-pollen on ovule development in stigmatic SI species will be required to address the question as to whether OSI is acting in tandem with stigmatic SI in the Polemoniaceae.

An outstanding difference between *I. aggregata* and other SI species within the Polemoniaceae is the observation that both cross- and self-pollen tubes growing within the style of *I. aggregata* do not deposit callose plugs or callose in their cell wall (Waser and Price, 1991; T. Sage, personal observations). In contrast, callose plugs and a callose cell wall is present in compatible pollen tubes in other species in the Polemoniaceae (Levin, 1975, 1993; Galen et al., 1989; Goodwillie et al., 2004). Amongst seed plants, callose deposition in pollen tubes is unique to angiosperms (Bell, 1995). The absence of callose in developing angiosperm pollen tubes is a rare trait and indicates that the pollen tubes of *I. aggregata* are physiologically different from those of other species within the taxa. Is the unique feature of pollen tube growth in *I. aggregata* important for understanding OSI as it compares to SI in other species within the Polemoniaceae? The complex and seemingly contradictory nature of SI in the Polemoniaceae demands that biology of SI in this family be examined in greater detail.

LITERATURE CITED

BARRETT, S. C. H. 1988. The evolution, maintenance, and loss of self-incompatibility systems. *In* J. Lovett Doust and L. Lovett Doust, [eds.], Plant reproductive ecology; patterns and strategies, 98–124. Oxford University Press, Oxford, UK.

BARRETT, S. C. H, L. D. HARDER, AND A. C. WORLEY. 1996a. The comparative biology of pollination and mating in flowering plants. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 351: 1271–1280.

BARRETT, S. C. H., D. G. LLOYD, AND J. ARROYO. 1996b. Stylar polymorphisms and the evolution of heterostyly in *Narcissus* (Amaryllidaceae). *In* D. G. Lloyd and S. C. H. Barrett, [eds.], Floral biology: studies on floral evolution in animal-pollinated plants, 339–376. Chapman and Hall, New York, New York, USA.

BELL, P. R. 1995. Incompatibility in flowering plants: adaptation of an ancient response. *Plant Cell* 7: 5–16.

BERNHARDT, P., T. L. SAGE, P. WESTON, H. ASUMA, M. LAM, L. B. THIEN, AND J. BRUHL. 2003. The pollination of *Trimenia moorei* (Trimeniaceae): floral volatiles, insect/wind pollen vectors, and stigmatic self-incompatiblity in a basal angiosperm. *Annals of Botany* 92: 445–458.

BITTENCOURT, N. S., P. E. GIBBS, AND J. SEMIR. 2003. Histological study of post-pollination events in *Sapthodea campanulata* Beauv. (Bignoniaceae), a species with late-acting self-incompatibility. *Annals of Botany* 91: 827–834.

BITTENCOURT, N. S., AND J. SEMIR. 2005. Late-acting self-incompatibility and other breeding systems in *Tabebuia* (Bignoniaceae). *International Journal of Plant Sciences* 166: 493–506.

BROYLES, S. B., AND R. WYATT. 1993. The consequences of self-pollination in *Asclepias exaltata*, a self-incompatible milkweed. *American Journal of Botany* 80: 41–44.

CHARLESWORTH, D. 1985. Distribution of dioecy and self-incompatibility in angiosperms. *In* J. J. Greenwood and M. Slatkin, [eds.], Evolution—essays in honour of John Maynard Smith, 237–268. Cambridge University Press, Cambridge, UK.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268.

CHENG, W., E. W. TALIERCIO, AND P. S. CHOUREY. 1996. The *miniature1* seed locus of maize encodes a cell wall invertase required for normal development of endosperm and maternal cells in the pedicel. *Plant Cell* 8: 971–983.

COOPER, D. C., AND R. A. BRINK. 1945. Seed collapse following matings between diploid and tetrapoloid races of *Lycopersicon pimpinellifolium*. *Genetics* 30: 376–401.

COPE, F. W. 1962. The mechanism of pollen incompatibility in *Theobroma cacao* L. *Heredity* 17: 157–182.

CRANE, M. B., AND A. BROWN. 1937. Incompatibility and sterility in the sweet cherry, *Prunus avium* L. *Journal of Pomology* 15: 86–99.

CRANE, M. B., AND W. J. C. LAWRENCE. 1929. Genetical and cytological aspects of incompatibility and sterility in cultivated fruits. *Journal of Pomology and Horticultural Science* 7: 276–301.

DE NETTANCOURT, D. 1977. Incompatibility in angiosperms. Springer-Verlag, Berlin.

DE NETTANCOURT, D. 1997. Incompatibility in angiosperms. *Sexual Plant Reproduction* 10: 185–199.

DE NETTANCOURT, D. 2001. Incompatibility and incongruity in wild and cultivated plants. Springer-Verlag, New York, New York, USA.

DULBERGER, R. 1964. Flower dimorphism and self-incompatibility in *Narcissus tazetta* L. *Evolution* 18: 361–363.

FRANKLIN-TONG, V. E., M. J. LAWRENCE, AND F. C. H. FRANKLIN. 1994. The molecular and cellular biology of gametophytic self-incompatibility in *Papaver rhoeas*. *In* E. B. Williams, R. B. Knox, and A. E. Clarke, [eds.], Genetic control of self-incompatibility and reproductive development in flowering plants, 42–64. Kluwer, Dordrecht, Netherlands.

GALEN, C., T. GREGORY, AND L. F. GALLOWAY. 1989. Costs of self-pollination in a self-incompatible plant, *Polemonium viscosum*. *American Journal of Botany* 76: 1675–1680.

GIBBS, P. E., AND M. BIANCHI. 1993. Post-pollination events in species of *Chorisia* (Bombacaceae) and *Tabebuia* (Bignoniaceae) with late-acting self-incompatibility. *Botanica Acta* 106: 64–71.

GIBBS, P. E., AND M. BIANCHI. 1999. Does late-acting self-incompatibility (LSI) show family clustering? Two more species of Bignoniaceae with LSI: *Dolichandra cynanchoides* and *Tabebuia nodosa*. *Annals of Botany* 84: 449–457.

GIBBS, P. E., P. E. OLIVEIRA, AND M. B. BIANCHI. 1999. Postzygotic control of selfing in *Hymenaea stigonocarpa* (Leguminosae-Caesalpinioideae), a bat-pollinated tree of the Brazilian cerrados. *International Journal of Plant Sciences* 160: 72–78.

GOODWILLIE, C. 1997. The genetic control of self-incompatibility *in Linanthus parviflorus* (Polemoniaceae). *Heredity* 79: 424–432.

GRANT, V., AND K. A. GRANT. 1965. Flower pollination in the phlox family. Columbia University Press, New York, New York, USA.

GRANT, V., AND D. H. WILKEN. 1986. Taxonomy of the *Ipomopsis aggregata* group. *Botanical Gazette* 147: 359–371.

HECHT, A. 1964. Partial inactivation of an incompatibility substance in the stigmas and styles of *Oenothera*. *In* H. F. Linskens [ed.], Pollen physiology and fertilization. North-Holland, Amsterdam, Netherlands.

HERRERO, M., AND A. ARBELOA. 1989. Influence of the pistil on pollen tube kinetics in peach (*Prunus persica*). *American Journal of Botany* 76: 1441–1447.

HERRERO, M., AND H. G. DICKINSON. 1980. Pollen tube growth following compatible and incompatible intraspecific pollinations in *Petunia hybrida*. *Planta* 148: 217–221.

HERRERO, M., AND M. GASCON. 1987. Prolongation of embryo sac viability in pear (*Pyrus communis*) following pollination or treatment with gibberellic acid. *Annals of Botany* 60: 287–293.

HESLOP-HARRISON, J. 1982. Pollen–stigma interactions and cross-incompatibility in the grasses. *Science* 215: 1358–1364.

KAPIL, R. N., AND S. C. TIWARI. 1978. The integumentary tapetum. *Botanical Review* 44: 457–490.

KENRICK, J., V. KAUL, AND E. G. WILLIAMS. 1986. Self-incompatibility in *Acacia retinodes*: site of pollen-tube arrest is the nucellus. *Planta* 169: 245–250.

KNOX, R. B. 1973. Pollen wall proteins: pollen–stigma interactions in ragweed and cosmos (Compositae). *Journal of Cell Science* 12: 421–443.

KOEHL, V. 2002. Functional reproductive biology of *Illicium floridanum* (Illiciaceae). Master's thesis, University of Toronto, Toronto, Ontario, Canada.

KOLTUNOW, A. M. 1993. Apomixis: embryo sacs and embryos formed without meiosis or fertilization in ovules. *Plant Cell* 5: 1425–1437.

LADOUX, T. 2004. Self-incompatibility in *Ipomopsis tenuifolia*. Ph.D. dissertation, Claremont Graduate University, Claremont, California, USA.

LEVIN, D. A. 1975. Gametophytic selection in phlox. *In* D. L. Mulcahy [ed.], Gamete competition in plants and animals. North Holland, Amsterdam, Netherlands.

LEVIN, D. A. 1993. S-gene polymorphism in *Phlox drummondii*. *Heredity* 71: 193–198.

LEWIS, G., AND P. GIBBS. 1999. Reproductive biology of *Caesalpinia calycina* and *C. pluviosa* (Leguminosae) of the cattanga of north-eastern Brazil. *Plant Systematics and Evolution* 217: 43–53.

LIPOW, S. R., AND R. WYATT. 2000. Single gene control of postzygotic self-incompatibility in poke milkweed, *Asclepias exalata* L. *Genetics* 154: 893–907.

LLOYD, D. G., AND M. S. WELLS. 1992. Reproductive biology of a primitive angiosperm, *Pseudowintera colorata* (Winteraceae) and the evolution of pollination systems in the Anthophyta. *Plant Systematics and Evolution* 181: 77–95.

MARTIN, F. M. 1959. Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technology* 34: 436–437.

MILLER, M. E., AND P. S. CHOUREY. 1992. The maize invertase-deficient *miniature-1* seed mutation is associated with aberrant pedicel and endosperm development. *Plant Cell* 4: 297–305.

O'BRIEN, T. P., AND M. E. MCCULLY. 1981. The study of plant structure: principles and selected methods. Termarcarphi, PTY., Melbourne, Victoria, Australia.

OFFLER, C. E., D. W. MCCURDY, J. W. PATRICK, AND M. J. TALBOT. 2002. Transfer cells: cells specialized for a special purpose. *Annual Review of Plant Biology* 54: 431–454.

O'NEILL, S. 1997. Pollination regulation of flower development. *Annual Review of Plant Physiology and Plant Molecular Biology* 48: 547–574.

OWENS, S. J. 1981. Self-incompatibility in the Commelinaceae. *Annals of Botany* 47: 567–581.

PIMIENTA, E., AND V. S. POLITO. 1982. Ovule abortion in 'Nonpareil' almond (*Prunus dulcis* [Mill.] D.A. Webb). *American Journal of Botany* 69: 913–920.

PIMIENTA, E., AND V. S. POLITO. 1983. Embryo sac development in almond (*Prunus dulcis* [Mill.] D.A. Webb) as affected by cross, self, and non-pollination. *Annals of Botany* 71: 469–479.

PONTIERI, V., AND T. L. SAGE. 1999. Evidence for stigmatic self-incompatibility, pollination induced ovule enlargement and transmitting tissue exudates in the paleoherb, *Saururus cernuus* L. (Saururaceae). *Annals of Botany* 84: 507–519.

POUND, L. M., B. PATTERSON, M. A. B. WALLWORK, B. M. POTTS, AND M. SEDGLEY. 2003. Pollen competition does not affect the success of self-pollination in *Eucalyptus globulus* (Myrtaceae). *Australian Journal of Botany* 51: 189–195.

PRICE, M. V., N. M. WASER, R. E. IRWIN, D. R. CAMPBELL, AND A. K. BRODY. 2005. Temporal and spatial variation in pollination of a montane herb: a seven-year study. *Ecology* 86: 2106–2116.

SAGE, T. L., R. BERTIN, AND E. G. WILLIAMS. 1994. Ovarian and other late-acting self-incompatibility. *In* E. B. Williams, R. B. Knox, and A. E. Clarke, [eds.], Genetic control of self-incompatibility and reproductive development in flowering plants, 116–140. Kluwer, Dordrecht, Netherlands.

SAGE, T. L., S. R. GRIFFIN, V. PONTIERI, P. DROBAC, W. W. COLE, AND S. C. H. BARRETT. 2001. Stigmatic self-incompatibility and mating patterns in *Trillium grandiflorum* and *Trillium erectum* (Melanthiaceae). *Annals of Botany* 88: 829–841.

SAGE, T. L., AND F. B. SAMPSON. 2003. Evidence for ovarian self-incompatibility as a cause of self-sterility in the primitive woody angiosperm, *Pseudowintera axillaris* (Winteraceae). *Annals of Botany* 91: 1–10.

SAGE, T. L., F. STRUMAS, B. COLE, AND S. C. H. BARRETT. 1999. Differential ovule development following self- and cross-pollination: the basis of self-sterility in *Narcissus triandrus* (Amaryllidaceae). *American Journal of Botany* 86: 855–870.

SAGE, T. L., AND B. D. WEBSTER. 1990. Development of aborting and nonaborting seeds of *Phaseolus vulgaris* L. *Botanical Gazette* 151: 167–175.

SAGE, T. L., AND E. G. WILLIAMS. 1991. Self-incompatibility in *Asclepias*. *Plant Cell Incompatibility Newsletter* 23: 55–57.

SAGE, T. L., AND E. G. WILLIAMS. 1995. Structure, ultrastructure, and histochemistry of the pollen tube pathway in the milkweed *Asclepias exaltata* L. *Sexual Plant Reproduction* 8: 257–265.

SATINA, S., J. RAPPAPORT, AND A. F. BLAKESLEE. 1950. Ovular tumors connected with incompatible crosses in *Datura*. *American Journal of Botany* 37: 576–586.

SEARS, E. R. 1937. Self-sterility in plants. *Genetics* 22: 130–181.

SEAVEY, S. R., AND K. S. BAWA. 1986. Late-acting self-incompatibility in angiosperms. *Botanical Review* 52: 195–218.

SPARROW, I. K., AND N. L. PEARSON. 1948. Pollen compatibility in *Asclepias syriaca*. *Journal of Agriculture Research* 77: 187–199.

WASER, N. M., AND M. L. FUGATE. 1986. Pollen precedence and stigma closure: a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. *Oecologia* 70: 573–577.

WASER, N. M., AND M. V. PRICE. 1991. Reproductive costs of self-pollination in *Ipomopsis aggregata* (Polemoniaceae): are ovules usurped? *American Journal of Botany* 78: 1036–1043.

WASER, N. M., AND C. F. WILLIAMS. 2001. Inbreeding and outbreeding. *In* C. W. Fox, D. A. Roff, and D. J. Fairbairn, [eds.], Evolutionary ecology: concepts and case studies. Oxford University Press, Oxford, UK.

WESCHKE, W., R. PANITZ, S. GUBATZ, Q. WANG, R. RADCHUK, H. WEBER, AND U. WOBUS. 2003. The role of invertases and hexose transporters in controlling sugar ratios in maternal and filial tissues of barley caryopses during early development. *Plant Journal* 33: 395–411.

WILLIAMS, E. G., V. KAUL, J. L. ROUSE, AND R. B. KNOX. 1984. Apparent self-incompatibility in *Rhododendron ellipticum*, *R. championae*, and *R. amamiense*: a post-zygotic mechanism. *Incompatibility Newsletter* 16: 10–11.

WOBUS, U., AND H. WEBER. 1999. Sugars as signal molecules in plant seed development. *Biological Chemistry* 380: 937–944.

ZINSELMEIER, C., J. BYEONG-RYONG, AND J. S. BOYER. 1999. Starch and the control of kernel number in maize at low water potentials. *Plant Physiology* 121: 25–35.