

POLLEN QUALITY LIMITS SEED SET IN *BURCHARDIA UMBELLATA* (COLCHICACEAE)¹

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In self-incompatible plants, interference by self pollen or genetically related pollen can potentially exacerbate pollen limitation, although this has rarely been demonstrated. We examined the breeding system, pollen limitation, and pollen interference using self- and cross- pollinations and pollen supplementations in *Burchardia umbellata*, an insect-pollinated lilioid monocot. Ovule fertilization and seed set were less following selfing than crossing (22 vs. 78% and 4 vs. 73%, respectively), indicating partial self-incompatibility. Flowers were partially protandrous, and flowers opened concurrently on plants potentially allowing self pollen interference. Natural seed set was pollen limited and varied within and among years, probably due to variation in flowering plant density. Interference by self or genetically related pollen caused pollen limitation as evidenced by increased seed set of bagged cross-pollinated plants compared to unbagged pollen-supplemented plants in two years. In 1996, both fertilization and seed set increased in response to cross-pollination, indicating that interference occurred in the style and ovary. In 1997, only seed set increased after cross-pollination indicating that interference occurred in the ovary. Inappropriate pollen deposition may contribute to pollen limitation more often than previously recognized and should select for floral traits that decrease deposition of self or related pollen.

Key words: *Burchardia umbellata*; Colchicaceae; pollen limitation; pollen quantity; self-incompatibility; self-pollen interference.

Many flowering plants rely on pollinators to deposit sufficient compatible pollen onto stigmas for ovule fertilization and seed production. Pollen limitation occurs when seed production is less than would be achieved if overall quantity or quality of pollen deposited onto stigmas were increased. Pollen quantity may be limiting if pollinators are rare, or if plants compete for the services of pollinators (Bierzychudek, 1981; Johnston, 1991; Groom, 1998). Pollen quality may be limiting, despite adequate pollination, if pollinators deposit on stigmas self or incompatible pollen, or deposit closely related pollen and early-acting inbreeding depression lowers seed set (Waser and Price, 1983, 1991a; Manasse and Pinney, 1991; Byers, 1995; Totland et al., 1998). In a recent survey, seed production was pollen limited in 62% of 258 species surveyed, indicating that pollen limitation is a common proximate cause of low seed set (Burd, 1994); other explanations for low seed:ovule ratios are provided by Bawa and Webb (1984). An important finding of many studies is that the occurrence or extent of pollen limitation varies within and among flowering seasons (Copland and Whelan, 1989; Dudash and Fenster, 1997). Quantifying the extent of pollen limitation and the relative importance of pollen quantity and pollen quality in limiting seed set are necessary to provide insights into the functional significance of floral traits and the selective factors involved in their evolution (Bawa and Webb, 1984; Lloyd and Webb, 1986; Bertin and Newman, 1993; Totland et al., 1998). Selection should favor traits that increase pol-

len receipt when pollen quantity is limiting, whereas traits that reduce deposition of self pollen and/or closely related pollen should be favored when pollen quality is limiting.

Pollen limitation is usually tested by supplementing plants that have been previously exposed to pollinators with excess cross pollen. If seed production of such plants is greater than control plants this is taken as evidence of pollen limitation (Zimmerman and Pyke, 1988; but see Bawa and Webb, 1984). However, prior or simultaneous deposition of self, incompatible, or closely related pollen by pollinators may interfere with the ability of plants to use available cross pollen, resulting in reduced seed set. In many species, especially those that are self-incompatible, substantial interference could result from selfing. Potential mechanisms of interference include clogging or blocking of stigma surfaces, stylar tissues, and ovular micropyles and fertilizing ovules that are later aborted due to late-acting self-incompatibility or inbreeding depression (Bawa and Opler, 1975; Ockendon and Currah, 1977; Seavey and Bawa, 1986; Galen, Gregory, and Galloway, 1989; Waser and Price, 1991b; Broyles and Wyatt, 1993; Ramsey, 1995). Under such conditions, standard tests will underestimate the full extent of pollen limitation. In some species, experimental application of self pollen before or simultaneously with cross pollen reduces seed set, indicating that self pollen interference may exacerbate pollen limitation under natural conditions (Galen, Gregory, and Galloway, 1989; Palmer, Travis, and Antonovics, 1989; Waser and Price, 1991b; Broyles and Wyatt, 1993; Ramsey, 1995; Barrett, Lloyd, and Arroyo, 1996). Other studies have failed to detect negative effects of self-pollination, and in such species self-pollen interference may be minimal (Shore and Barrett, 1984; Nishihiro and Washitani, 1998). Few studies, however, have assessed the magnitude of interference that occurs under natural pollination conditions (but see de Jong et al., 1992; Ramsey, 1995).

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Opportunities for self pollen to compete with cross pollen for access to stigma surfaces, stylar tissues, or ovules will be influenced by a variety of floral traits including dichogamy and floral display size. Dichogamy influences the likelihood of autogamous self-pollination by separating in time male and female functions within flowers (Lloyd and Webb, 1986; Palmer, Travis, and Antonovics, 1989). The widespread occurrence of dichogamy in self-incompatible species has led to the suggestion that this trait has evolved to reduce self-pollen interference rather than to promote outbreeding (Lloyd and Webb, 1986; Bertin and Newman, 1993). Aspects of floral display including the number and arrangement of flowers, as well as their individual attractiveness, influence the likelihood of geitonogamous self-pollination (de Jong et al., 1992; Snow et al., 1996). Although selection can act upon floral displays to reduce geitonogamy, such changes are also likely to reduce pollinator attraction and levels of cross-pollination (Klinkhamer and de Jong, 1993). Interference due to the deposition of genetically related pollen depends upon the degree of genetic structuring in populations. Pollinators often move short distances between plants and when fine-scale genetic structure exists, pollen transfer can occur between related individuals (Waser and Price, 1983, 1991a).

In this study, we examine the extent, frequency, and causes of pollen limitation in the perennial herb *Burchardia umbellata* R. Br. (Colchicaceae). Specifically, we examine the importance of pollen quantity and pollen quality by assessing pollen deposition on stigmas and by comparing seed set, ovule fertilization, and seed abortion of open-pollinated, pollen-supplemented, and cross-pollinated plants in which selfing was prevented. Differences in fertilization and seed set between open-pollinated plants and pollen-supplemented plants could be due to pollen quantity and/or pollen quality. Differences between pollen-supplemented and cross-pollinated plants indicate interference due to pollen quality. To determine the likelihood of selfing under natural conditions, we also examine the degree to which flowers are self-fertile and protandrous and assess floral display size.

MATERIALS AND METHODS

Study site and species—*Burchardia umbellata* occurs in grassland, heathland, and dry sclerophyll forest in southern Australia. We studied a *B. umbellata* population in a disturbed open *Eucalyptus* forest with grassy understorey near Mansfield in Victoria (37°03' S; 146°05' E) from 1996 to 1998. The population contained >1000 plants, but not all flowered in each year. Plant flowering in part depends on winter and spring rainfall, and flowering plant density was ranked as low, medium, and high in 1996, 1997, and 1998, respectively.

In the glasshouse, plants can clone from corms; 31 of 220 plants produced clones over 3 yr (M. Ramsey, unpublished data). Cloning, however, may be uncommon under natural conditions. Clones are found in clumps, whereas during periods of very high flowering plants closest to each other were well spaced (~10–20 cm). In the field, plants bloom in spring, from October to November. Flowering plants have a corm, tuberous roots, one or two basal annual leaves, and a terminal umbel with two to nine white hermaphroditic flowers. Flowers have six tepals (5–8 mm long) in one whorl, are sweetly scented, and produce nectar. Anthers (6) are extrorse and dehisce by longitudinal slits. The stigma and anthers are well separated (~3–4 mm), and flowers do not autonomously self pollinate. Flowers are visited by native solitary bees, in-

roduced honey bees (*Apis mellifera*), flies, and beetles. Some floral buds abort, but most flowers produce fruits. Fruits are capsules that contain ~15 seeds.

Self-fertility—We assessed self-fertility of plants by randomly assigning the first and second flowers to open on each of 18 umbels to either cross- or self-pollination treatments. Both treatments on the same umbel does not affect self seed set (M. Ramsey, unpublished data). Crossed flowers were emasculated as they opened and pollinated 3 d later with pollen from three to five plants ~10 m distant. Selfed flowers were pollinated 3 d after they opened with pollen from a newly opened flower on the same plant. After flowers were pollinated other flowers and buds were removed. Pollinators were excluded from flowering umbels with fabric mesh bags. Fruits were harvested 4–5 wk after pollinations, and the number of ovules, aborted seeds, and seeds were counted. Aborted seeds were shrivelled and 25–75% the size of seeds. The number of aborted seeds was probably underestimated since embryos that aborted early in development could not be detected. We compared percentage seed set, ovule fertilization, and seed abortion using random block ANOVAs, with plants and pollination treatment as random and fixed factors, respectively. To reduce Type I errors, factors were tested for significance by a sequential Bonferroni procedure with an overall $\alpha = 0.05$ (Rice, 1989).

We assessed pollen:ovule ratios as conservative indicators of breeding systems and sexual allocation (Cruden, 1977; Charnov, 1987). To assess pollen production, undehisced anthers of first flowers from 53 umbels were collected into vials. As anthers dehisced 0.5 mL of lactophenol was added and the number of pollen grains counted in four haemocytometer grids. We counted the number of ovules in the same flowers with a dissecting microscope.

Variation in natural seed set—We assessed seed set at peak flowering in 1996, and at early and peak flowering in 1997 and 1998. Percentage seed set was assessed on the first flower to open on plants. In 1996, flowers were marked on 24 plants. In early 1997, flowering was poor and kangaroos ate some umbels, so only ten plants could be used. For other periods, flowers were marked on 30 plants. Seed set at peak flowering was compared among years with a one-way ANOVA. We also examined the effect on seed set of flowering time (early vs. peak, fixed factor) and year (1997 vs. 1998, random factor) with a two-way model III ANOVA.

Pollen limitation and self-pollen interference—We examined variation in pollen limitation and the occurrence of self-pollen interference by randomly assigning plants at peak flowering in 1996 and 1997 to the following treatments: (1) open pollination—plants were left untouched to assess natural seed set; (2) pollen supplementation—flowers were left unbagged and open to pollinators and were cross-pollinated 3 d after they opened; and (3) cross-pollination—newly opening flowers were emasculated, bagged with mesh to exclude pollinators, and cross-pollinated 3 d later. Cross pollen was from three to five plants at least 10 m distant. Stigmas on open-pollinated and pollen-supplemented plants could receive self pollen autogamously or geitonogamously, genetically related pollen from nearby plants, and unrelated cross pollen. Bagged + cross-pollinated plants received only cross pollen. It is unlikely that the use of bags on cross-pollinated plants provided beneficial or protective effects. Bags were removed when flowers wilted and no insects were observed to damage ovaries of unbagged flowers. Sample sizes varied between 21 and 32 plants.

In 1996, all flowers on plants were treated. In 1997, the first two or three flowers to open on plants were treated; remaining flowers and buds were removed when pollinations were completed. We harvested fruits 4–5 wk after flowering and counted the number of ovules, aborted seeds, and seeds. For each trait, we used a two-way model III ANOVA to examine for interactions between treatment and years, fixed and random factors, respectively. To reduce Type I errors, interactions were

tested for significance by a sequential Bonferroni procedure at an overall $\alpha = 0.10$ (Rice, 1989).

We assessed the efficacy of using a subsample of fruits on plants to estimate whole plant seed set. On the 1996 experimental plants, we assessed seed set of the first two flowers to open and total seed set of plants. We used a model II geometric mean regression to calculate the slope of the line between percentage seed set from the fruits and the whole plants. If the slope equals 1.0, seed set of the two fruits is a good predictor of whole-plant seed set. We also determined the effect of removing floral buds on seed set of the remaining flowers by assigning 30 plants to a removal treatment and 25 plants to an unmanipulated control. On removal plants, all buds except the first two were removed when flowering was completed. Percentage seed set was compared with a one-way ANOVA.

Pollen deposition—We compared pollen deposition and ovule production in 1996 and 1997. At peak flowering, newly opened flowers on different plants were marked and harvested 5 d later near the end of floral life (1996, $N = 26$; 1997, $N = 20$). Stigmas were placed onto small cubes of glycerin jelly with basic fuchsin and squashed under a coverslip. The total number of pollen grains on stigmas was counted at 40 \times magnification. We did not distinguish between germinated and ungerminated grains. The number of ovules per flower was assessed from 40 open-pollinated flowers in each year. We compared pollen deposition with ovule production in each year using an unpooled two-sample t test for unequal variances.

Dichogamy—Self-pollen interference caused by within-flower selfing is potentially related to the degree of overlap between male and female functions. We examined patterns of anther dehiscence, pollen longevity, onset and duration of stigma receptivity, and floral longevity in the laboratory. The temperature in the laboratory ($21.5^\circ \pm 0.5^\circ$ C) was similar to field temperatures during the day, but was warmer at night. Umbels ($N = 14$) were placed in water. The first flower to open on each was examined every 12 h for 8 d, and the number of dehiscent anthers and condition of stigmas and petals were recorded. Unpollinated flowers in the laboratory provide conservative estimates of floral biology since pollination or environmental conditions in the field may alter individual traits.

We assessed pollen longevity by placing pollen of known age on 10% sucrose germination medium. Dehiscent anthers were removed from flowers, and 2 h later, one newly dehiscent anther on each of five flowers on different umbels was marked. Pollen was sampled from each anther with a dissecting needle at 0, 12, 24, 36, and 48 h later, placed on a drop of medium on a microscope slide, and incubated on moist filter paper in a petri dish. After 10 h, a drop of acetocarmine was added and a coverslip was applied. Transects across slides were viewed at 40 \times magnification and the number of ungerminated and germinated grains counted. The proportion of germinated pollen grains was calculated from 200 grains in each sample.

We assessed stigma receptivity by pollinating flowers of known age and indirectly assessing pollen germination. Open flowers on umbels were removed. About 6 h later, 30 newly opened flowers on different umbels were emasculated, allocated to 0-, 24-, 48-, 72-, 96- and 120-h treatments, and lightly cross-pollinated with fresh pollen at the appropriate time. After 6 h, stigmas were placed in acetocarmine for 10 min. Stigmas were transferred to small cubes of clear glycerin jelly and squashed under a coverslip. Germinated grains do not stain, whereas ungerminated grains stain red. Pollen grains were counted at 40 \times magnification and the proportion of stained grains calculated.

We indirectly examined the reproductive consequences of overlap between pollen longevity and stigma receptivity. In the field, we assessed whether fresh pollen deposited on nonreceptive stigmas shortly after flowers opened was capable of fertilizing ovules when stigmas later became receptive. At 0700 before insect pollinators became active, we removed undehiscent anthers of newly opening flowers. Umbels were

TABLE 1. Percentage seed set, fertilization, and abortion for cross- and self-pollinated flowers. Data are means \pm 1 SE and were analyzed with a random block ANOVA with plants as a random factor ($N = 18$). For all parameters, pollination treatment was significant based on a sequential Bonferroni test of tablewide significance level $\alpha = 0.05$.

Parameters	Cross	Self	Pollination $F_{(1,17)}$	Plant $F_{(17,17)}$
Seed set (%)	73.3 \pm 4.1	4.4 \pm 2.0	158.45***	0.45 ^{NS}
Fertilization (%)	77.7 \pm 4.2	22.0 \pm 3.9	64.17***	0.62 ^{NS}
Abortion (%)	5.8 \pm 1.5	80.6 \pm 6.6	102.91***	1.34 ^{NS}

^{NS} > 0.05 , *** $P < 0.001$.

bagged and assigned to treatments in which flowers were cross-pollinated either 4 h later when flowers had fully opened or 3 d after they had opened. Stigmas were not receptive 4 h after flowers opened but were fully receptive 3 d after flowers opened. If pollen becomes inviable before the onset of stigma receptivity, then few or no seeds would be produced in the 4-h treatment, whereas maximal seeds would be produced in the 3-d treatment. We used cross pollen rather than self pollen to assess the potential for self-pollen interference, because this allowed us to examine the effects of overlap between stigma receptivity and pollen longevity on seed set independently of the confounding effects of self-incompatibility and inbreeding depression. Percentage seed set was compared using a one-way ANOVA.

Floral display size—When more than one flower is open per plant, geitonogamy and self-pollen interference can occur. We assessed floral display size in the laboratory by monitoring 14 umbels with unopened flowers and counting the number of open flowers each day for 8 d. We classified open flowers as those that had at least one dehiscent anther, white stigmas, and white unwithered petals. The schedules of flower opening in the laboratory and field are similar. Consequently, we examined 86 plants in the field, counting the total number of flowers and estimating only the maximal number of open flowers per plant.

Analyses—We calculated seed set as the proportion of ovules that developed into seeds. Ovule fertilization was calculated as (aborted seeds + seeds) / (ovules + aborted seeds + seeds), and seed abortion was calculated as (aborted seeds) / (aborted seeds + seeds). Percentages and counted data were arcsine and square-root transformed, respectively, which improved homogeneity of variances and normality as indicated by Levene's tests and normal probability plots. In the two-way model III ANOVAs, all interactions were significant and we further analyzed the effects of the fixed factors using one-way ANOVAs separately for each year, testing for significance with sequential Bonferroni procedures at an overall $\alpha = 0.05$ (Rice, 1989; Underwood, 1997). To reduce Type I errors, we also used $\alpha = 0.01$ to test significance of Tukey a posteriori tests. Means \pm 1 SE are given. Minitab 10Xtra (Minitab Inc, State College, Pennsylvania) was used for analyses.

RESULTS

Self-fertility—Percentage seed set, ovule fertilization, and seed abortion in self- or cross-pollinated flowers differed significantly. Selfed seed set and fertilization were 69 and 55%, less than following crossing, respectively, but abortion was 75% greater (Table 1). Among-plant variation was not significant in any of the analyses (Table 1). Mean relative self-fertility (RSF = self/cross seed set) was low (0.07 ± 0.03), indicating partial self-incompatibility. Variation in RSF was pronounced, ranging from 0.0 to 0.50.

The mean numbers of pollen grains and ovules per flower were $356\,993 \pm 18\,277$ and 31.2 ± 0.8 , respec-

TABLE 2. Percentage seed set of open-pollinated plants during peak flowering in 1996, and early and peak flowering in 1997 and 1998. Flowering densities, given in parentheses, could be ranked as low, medium, or high. Data are means \pm 1 SE, calculated from the first flowers to open on plants. Seed set was less during peak flowering in 1996 and early flowering in 1997 than during other periods ($P < 0.01$).

Sample period (density)	Seed set (%)	N
Peak 1996 (low)	32.5 \pm 3.0	24
Early 1997 (low)	27.6 \pm 5.4	10
Peak 1997 (medium)	49.4 \pm 2.4	30
Early 1998 (medium)	46.2 \pm 2.2	30
Peak 1998 (high)	47.2 \pm 2.0	30

tively. The pollen:ovule ratio was 11 442, indicating that the breeding system is most likely outcrossing (Cruden, 1977; Charnov, 1987). Pollen production was not correlated with ovule production within flowers ($r = + 0.20$; $P > 0.05$; $N = 53$).

Variation in natural seed set—Percentage seed set at peak flowering differed significantly among years (Table 2; $F_{2,61} = 9.06$, $P = 0.000$). Seed set was less in 1996 than in 1997 and 1998 (Tukey tests, $P < 0.01$). The interaction was significant in the two-way ANOVA, indicating that the effects of flowering time and year on seed set were not independent ($F_{1,96} = 16.44$, $P = 0.000$). Flowering time affected seed set in 1997 and was less during early compared to peak flowering (Table 2; one-way ANOVA, $F_{1,38} = 19.14$, $P = 0.000$). In 1998, early and peak seed set did not differ (Table 2; one-way ANOVA, $F_{1,58} = 0.11$, $P = 0.74$).

Pollen limitation and self-pollen interference—All interactions were significant in the two-way ANOVAs, indicating that the effects of pollination treatment and year on the parameters were not independent (seed set: $F_{2,144} = 9.16$, $P = 0.000$; fertilization: $F_{2,144} = 4.38$, $P = 0.014$; abortion: $F_{2,144} = 3.74$, $P = 0.026$; all $P < 0.08$ using sequential Bonferroni procedure). Results of one-way ANOVAs within years are given in Fig. 1. In 1996, but not 1997, seed set following pollen supplementation was significantly greater than open pollination. In both years, cross-pollination significantly increased seed set compared to pollen supplementation (Fig. 1a). Ovule fertilization following cross-pollination was also significantly increased compared to pollen supplementation, which was greater than open pollination. In 1997, ovule fertilization did not differ among treatments (Fig. 1b). Seed abortion in open-pollinated plants was significantly greater than pollen-supplemented plants in 1996, but not in 1997. In both years, seed abortion was significantly great-

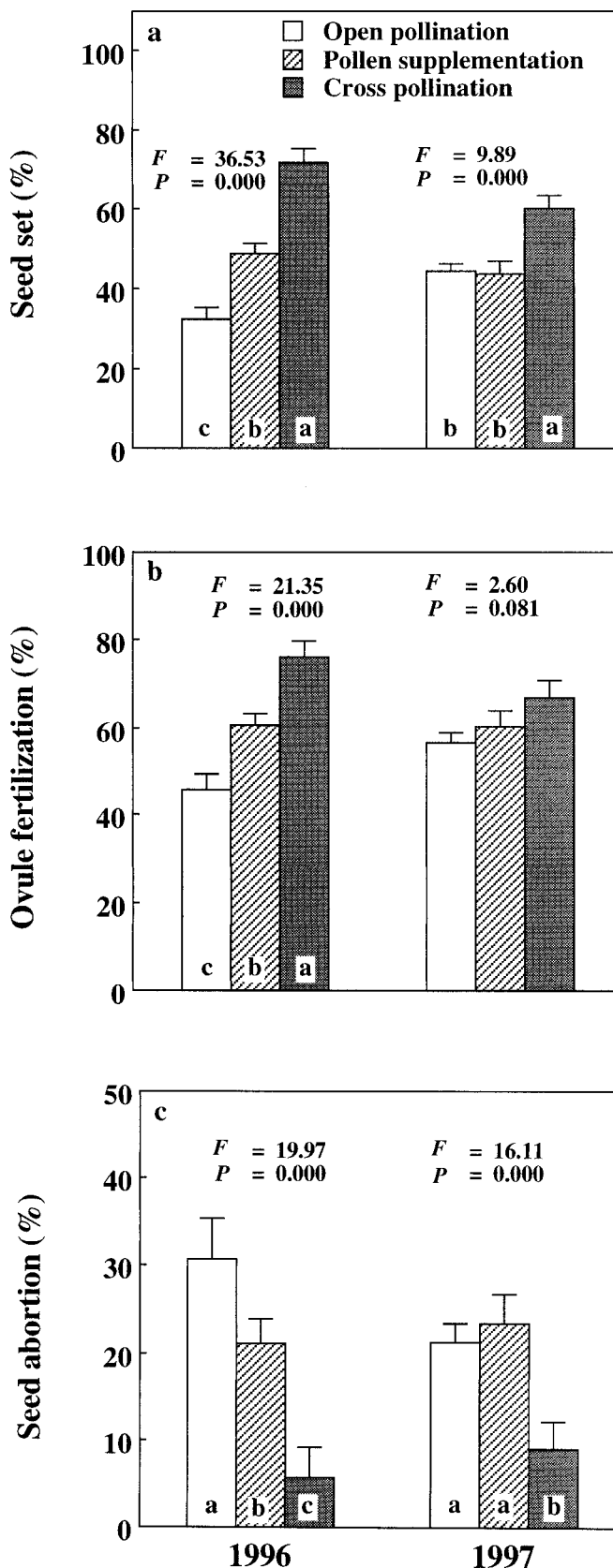


Fig. 1. Mean (\pm 1 SE) percentage seed set (a), ovule fertilization (b), and seed abortion (c) following open pollination, pollen supplementation, and cross-pollination in 1996 and 1997. F ratios and probabilities from one-way ANOVAs are given (1996: $df = 2,69$; 1997: $df = 2,75$). For all parameters, pollination treatment was significant based on a sequential Bonferroni test of tablewise significance level $\alpha = 0.05$. Within years, columns with the same letter do not differ significantly (Tukey tests, $P > 0.01$).

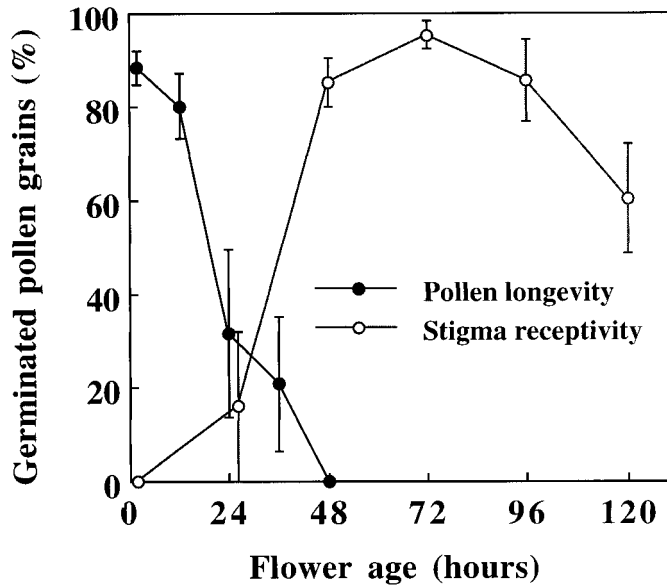


Fig. 2. Pollen longevity and stigma receptivity with time after flower opening using cut plants that were maintained in the laboratory. For pollen longevity, pollen of a known age was germinated on sucrose medium. For stigma receptivity, fresh pollen was germinated on stigmas of a known age. Mean (± 1 SE) percentages of germinated pollen grains are given ($N = 5$).

er following pollen supplementation than following cross-pollination (Fig. 1c).

Seed set assessed from the first two fruits on plants was a good predictor of whole-plant seed set. The slope of the model II regression between percentage seed set of the two fruits and whole plants did not differ significantly from 1.0 ($y = 4.49 + 0.91x$, $P = 0.125$, $r^2 = 0.83$). Also, removal of nonexperimental flowers from plants did not affect seed set of those that remained (removal, 52.5 ± 2.4 vs. nonremoval, 47.7 ± 1.5 ; $F_{1,53} = 2.70$, $P = 0.106$).

Pollen deposition—The number of pollen grains deposited on stigmas was significantly greater than the number of ovules per flower in each year (1996— 52.0 ± 7.3 vs. 29.5 ± 1.2 , $t_{25} = 3.16$, $P = 0.004$; 1997— 116.3 ± 11.8 vs. 29.2 ± 1.1 , $t_{19} = 11.79$, $P = 0.000$). The ratio of deposited pollen grains to ovules was 1.7 and 4.0 in 1996 and 1997, respectively. Pollen deposition was two-fold greater in 1997 than 1996 ($F_{1,44} = 35.5$, $P = 0.000$).

Dichogamy—Flowers were partially protandrous. Pollen was presented before stigmas became receptive, but some overlap occurred between male and female functions, indicating opportunities for pollinator-mediated autogamy (Fig. 2). Anthers dehiscence sequentially within flowers starting on the first day that flowers opened. On average, all anthers had dehiscence 32 h after flowers opened. Stigmas of newly opened flowers (<24 h old) were white, and lobes were less reflexed and drier than flowers that had been open for longer. Stigmas turned brown after 5–6 d, and petals started to wither and turn brown. All flowers on umbels had opened after 5.4 ± 0.3 d ($N = 14$).

About 88% of pollen grains germinated when anthers

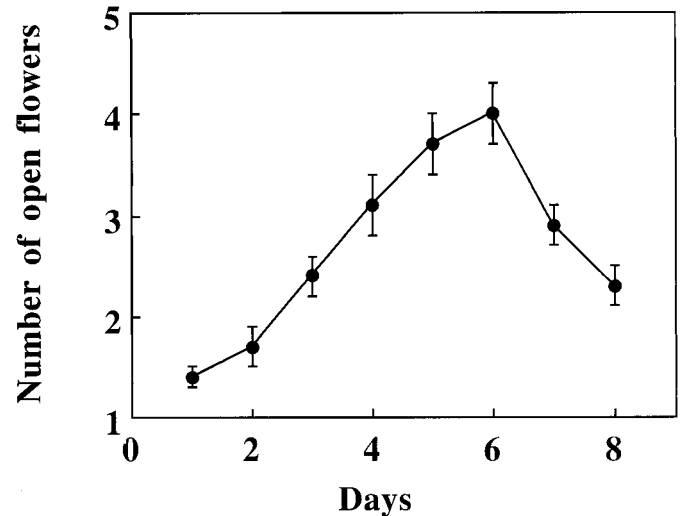


Fig. 3. The mean (± 1 SE) number of open flowers per plant with time after the first flower opened on umbels using cut plants that were maintained in the laboratory ($N = 14$).

first dehiscence and viability remained high for at least 12 h after dehiscence. About 30% of pollen germinated after anthers had been open for 24 h. After 48 h, pollen was completely inviable (0% germination; Fig. 2).

All pollen grains on stigmas of newly opened flowers were stained, indicating that they had not germinated and stigmas were unreceptive (Fig 2). About 80% of pollen was stained on 24-h old stigmas. Those that were unstained were on one stigma, indicating that most stigmas were unreceptive. At 48–96 h, 85–95% of pollen was unstained, and all stigmas were receptive. At 120 h, pollen staining declined to 60%, indicating that stigmas were becoming unreceptive.

In the 4 h vs. 3 d experiment, percentage cross seed set was substantial when flowers were pollinated shortly after they opened, but was significantly less than flowers pollinated after 3 d ($51.0 \pm 5.7\%$ vs. $71.9 \pm 3.6\%$; $F_{1,35} = 10.06$, $P = 0.003$). Thus stigmas became receptive before pollen became inviable. This indicates the potential for ovule pre-emption and self-pollen interference caused by pollinators depositing self pollen onto stigmas of newly opened flowers.

Floral display size—The pattern of flower opening within umbels was similar in the laboratory and the field and indicated opportunities for geitonogamy. In the laboratory, the number of open flowers per plant increased from 1.4 on the first day of flowering to 4.0 after 6 d. Thereafter the number of open flowers declined as flowers senesced (Fig. 3). In the field, the mean number of flowers per plant was 6.3 ± 0.1 and a maximum of 4.3 ± 0.1 flowers (68%) were open concurrently.

DISCUSSION

Burchardia umbellata was partially self-incompatible, and natural seed set was limited by both pollen quantity and pollen quality. Seed set of bagged cross-pollinated plants (cross-pollination) was greater than that of unbagged cross-pollinated plants (pollen supplementation) for

2 yr. Reduced seed set of pollen-supplemented plants was due to interference caused by the quality of pollen that was deposited naturally by pollinators. Excess cross pollen was applied by hand in both treatments, but pollen-supplemented plants were exposed to visits by insect pollinators prior to hand pollinations. Flowers do not autonomously self-pollinate, and interference was probably due to pollinators depositing self pollen or depositing pollen from genetically related individuals, probably near neighbors. This is one of only a few studies demonstrating adverse effects of interference due to pollen quality under natural conditions (Snow, 1982; Waser and Price, 1991b; de Jong et al., 1992; Morse, 1994; Ramsey, 1995).

Natural seed set varied substantially within and among years and was probably related to differences in flowering plant density. During low-flowering periods seed set was correspondingly low, ~17% less than in high-flowering periods (31 vs. 48%). Pollinator visitation was probably also less during periods of poor flowering as evidenced by the twofold reduction in pollen deposition at peak flowering in 1996 compared to 1997. Low flowering densities and small flowering patches often attract few pollinators, resulting in pollen limitation (Sih and Baltus, 1987; Groom, 1998). Pollen deposition exceeded the number of ovules during low flowering in 1996 (1.7 grains/ovule), but pollen quantity may have been limiting if several pollen grains per ovule are required for seed set. Alternatively, pollen quality may have been limiting if stigmatic pollen loads comprised mostly self pollen, leaving some ovules unfertilized.

Interference by inappropriate pollen caused pollen limitation in *B. umbellata*. In 1996, fertilization and seed set were increased by 15 and 16%, respectively, following pollen supplementation, and were further increased by 16 and 23% following cross-pollination. This indicates that seed set was limited by the quantity and/or quality of pollen that was deposited, and also that the deposition of inappropriate pollen by pollinators resulted in interference. In 1997, neither fertilization nor seed set increased significantly following pollen supplementation, although seed set was increased by 16% following cross-pollination. Thus in 1997 seed set was limited primarily by pollen quality and interference effects. These results highlight the importance of using bagged + cross-pollination treatments when testing for pollen limitation. The standard method of supplementing flowers with excess cross pollen does not account for possible interference due to the quality of pollen that is deposited naturally. There is increasing evidence that seed set is frequently pollen limited in plant populations (Burd, 1994), and interference resulting from inappropriate pollen deposition may contribute to pollen limitation much more than previously recognized.

In *B. umbellata*, flowers do not autonomously self-pollinate, and the deposition of inappropriate pollen and interference was probably caused by selfing due to pollinator mediated autogamy and geitonogamy, and by matings between genetically related plants, biparental inbreeding. For interference to reduce seed set, fewer seeds must be produced following inappropriate pollination than appropriate pollination (hereafter, crossing) and inappropriate pollen must compete with cross pollen for access to stigmas, styles, or ovules. Inappropriate pollen

interference is expected to reduce fitness more in self-incompatible plants than self-compatible plants, particularly when ovules are pre-empted by self pollen (Waser and Price, 1991b; Snow et al., 1996).

We found that *B. umbellata* was partially self-incompatible; self vs. cross ovule fertilization, seed set, and seed abortion were 22 vs. 78%, 4 vs. 73%, and 81 vs. 6%, respectively. Thus, selfed seed set was prevented by both pre- and postzygotic mechanisms as evidenced by reduced fertilization and increased abortion following selfing. Such differences are commonly attributed to genetically controlled self-incompatibility (SI) that is expressed in either the stigma, style, or ovary (Seavey and Bawa, 1986; Richards, 1997). In *B. umbellata*, the self-fertilization rate of 22%, the high proportion of aborted selfed seeds (81%) occurring at different developmental stages, and the wide range of selfed seed sets among plants are indicative of partial or weak SI and early-acting inbreeding depression (Manasse and Pinney, 1991; Ramsey, Prakash, and Cairns, 1993, and references within). The partially effective SI could act either prezygotically in the stigma and/or style, or act in the ovules. With the latter, late-acting SI, ovules are pre-empted by selfing and interference effects would be more pronounced than if SI is predominantly prezygotic. Studies in *B. umbellata* that examine pollen tube growth, ovule penetration, seed set, and seed genotype under different pollination regimes are now needed to determine the relative importance of pre- and postzygotic mechanisms in preventing selfing (Waser and Price, 1991b; Seavey and Carter, 1994; Ramsey, 1995; Cruzan and Barrett, 1996).

Self-pollen interference could occur via two general mechanisms in *B. umbellata*. First, self-pollen grains may clog stigmas preventing cross pollen from germinating or self-pollen tubes may clog styles preventing cross-pollen tubes from growing through stylar transmitting tissues (Ockendon and Currah, 1977; Galen, Gregory, and Galloway, 1989). Second, self-pollen tubes may block ovular micropyles or fertilize ovules that are subsequently aborted rendering them unavailable for cross-fertilization (Waser and Price, 1991b; Seavey and Carter, 1994; Ramsey 1995). Self-pollen interference caused by the physical clogging of stigmas probably has a negligible effect in *B. umbellata* since overall deposition relative to the stigmatic area would have been low (52–116 pollen grains per stigma). In contrast, some interference occurred in the style, as evidenced by lower fertilization in pollen-supplemented plants compared to cross-pollinated plants in 1996. Considerable interference occurred in the ovary. Seed abortion was substantially greater in open-pollinated and pollen-supplemented plants than in cross-pollinated plants in both years, indicating that ovule pre-emption occurred under natural conditions and was probably due in part to self-fertilization of ovules. Self pollen reduces seed set by pre-empting ovules in other species, including *Blandfordia grandiflora* (Ramsey, 1995), *Ipomopsis aggregata* (Waser and Price, 1991b), *Asclepias exaltata* (Broyles and Wyatt, 1993), and *Narcissus triandrus* (Barrett, Lloyd, and Arroyo, 1996).

Within flowers, protandry is the presentation of pollen before stigmas become receptive. By reducing overlap between male and female functions, protandry reduces autogamous self-pollination and self-pollen interference

(Lloyd and Webb, 1986; Bertin and Newman, 1993). In *B. umbellata*, flowers were incompletely protandrous and thus only partially effective in reducing within-flower selfing mediated by pollinators. Anthers dehisced sequentially over a 32-h period, pollen was viable for 36–48 h, and stigmas started to become receptive 24–48 h after flowers opened. Hence, pollen from the last anthers to dehisce would have been viable when stigmas became receptive. In addition, when newly opened flowers were pollinated with fresh pollen, seed set was substantial (51%), indicating that pollen can adhere to nonreceptive stigmas and remain viable until stigmas become receptive. Because protandry is incomplete and pollen longevity and stigma receptivity overlap in *B. umbellata*, pollinator-mediated autogamy could result in self-pollen interference. Some autogamy probably occurs since flowers lack floral specializations and are pollinated by unspecialized insect visitors, which may be more likely to deposit self pollen than specialist visitors (Lloyd and Schoen, 1992).

Sequential flower opening and variation in attractiveness between new and old flowers can potentially reduce geitonogamy (Klinkhamer and de Jong, 1993; Snow et al., 1996). In *B. umbellata*, flowers opened sequentially within umbels, but male-stage flowers co-occurred with female-stage flowers, providing opportunities for geitonogamy and self-pollen interference. Also, the nonspecialist pollinators did not discriminate between newly opened male-stage flowers and older female-stage flowers (M. Ramsey, Australian National University, personal observations). Plants produced a mean of 6.3 flowers, of which about four were open when floral display size was maximal. Geitonogamy can cause severe interference even in species with small floral displays (Snow et al., 1996, and references within), and this effect is likely to be exacerbated when a large proportion of the flowers are open concurrently, such as occurs in *B. umbellata*.

Biparental inbreeding may be of considerable importance in causing interference by the deposition of inappropriate pollen (Waser and Price, 1983). In *B. umbellata*, fine-scale genetic structure within populations and biparental inbreeding is likely since seeds lack obvious dispersal mechanisms and unspecialized pollinators move short distances between plants. Such inbreeding could result in interference either by ovule pre-emption and subsequent abortion due to inbreeding depression or by self-incompatibility effects such as stylar blocking. The genetic determination of self-incompatibility in *B. umbellata* is unknown. However, the most common system, gametophytic SI, is only partially effective in preventing matings between closely related individuals if both incompatibility alleles are not shared (Richards, 1997). Pollen from closely related plants that share one SI allele by descent will be 50% compatible, and have a high probability of possessing some of the same deleterious genes. Matings between such plants would result in ovule fertilization and abortion of those zygotes that are homozygous for shared deleterious genes. The remaining pollen would be incompatible and could contribute to interference in a similar manner to that of self pollen. Other plants could be fully self-compatible but genetically related, and interference would be due to inbreeding depression. We did not explicitly examine whether selfing

or biparental inbreeding caused interference. Differences in fertilization, seed set, and seed abortion between pollen-supplemented and cross-pollinated plants represent the combined effects of selfing and biparental inbreeding and were pronounced in our study.

In *B. umbellata*, plants could potentially reduce self-pollen interference by increasing the time between anther dehiscence and stigma receptivity to reduce autogamy, and by decreasing the number of flowers open concurrently to reduce geitonogamy. A more discriminating SI system could also reduce interference by self pollen and, to a lesser extent, biparental inbreeding. However, geitonogamy is a consequence of restricted pollinator foraging, and may be an unavoidable cost of requiring a large floral display to attract pollinators (Lloyd and Schoen, 1992; Snow et al., 1996). Biparental inbreeding is also a consequence of restricted pollinator foraging and limited seed dispersal. Selection to reduce interference may be limited by genetic constraints, trade-offs between different functions, and selection at other life-cycle stages.

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