BRIEF COMMUNICATION

Self-pollen interference is absent in wild radish (*Raphanus raphanistrum*, Brassicaceae), a species with sporophytic self-incompatibility[1]

Vanessa A. Koelling[2] and Keith Karoly

Biology Department, Reed College, Portland, Oregon 97202-8199 USA

Explaining the diversity of mating systems and floral forms in flowering plants is a long-standing concern of evolutionary biologists. One topic of interest is the conditions under which self-pollination can interfere with seed set for flowering plants with a self-incompatibility system. We investigated the effect of self-pollen interference for wild radish, *Raphanus raphanistrum*, which has sporophytic self-incompatibility. We performed pollinations and determined seed set for plants grown in the greenhouse, using pollen mixtures representing either self- with outcross-pollen or outcross-pollen alone. Stigmas were collected for a subset of pollinated flowers to determine the number of pollen grains applied. Average seed set for the self/cross (5.13 seeds/pollination) and cross treatments (5.09 seeds/pollination) did not differ significantly. Stigmatic pollen loads averaged around 700 grains, an amount close to observed natural pollen loads on *R. raphanistrum*. We concluded that for *R. raphanistrum* in natural populations, self-pollen is unlikely to interfere with outcross-pollen success. This study is the first to investigate effects of self-pollen interference on seed set for a homomorphic species with sporophytic self-incompatibility where rejection occurs at the stigmatic surface.

Key words: *Raphanus raphanistrum*; self-pollination; sporophytic self-incompatibility; stigma clogging.

Flowering plants possess an extraordinary diversity in their mating systems and floral structures that has long begged explanation by evolutionary biologists. Self-pollen deposition is thought to have been a selective mechanism contributing to the evolutionary diversity of floral form (Barrett, 2002). For obligately outcrossing, hermaphroditic flowering plants, self-pollen deposition has the potential to affect male fitness through pollen discounting (Harder and Wilson, 1998), while female fitness could be affected through self-pollen's interference with outcross-pollen at the stigmatic surface, in the stylar region, or at the point of fertilization. The avoidance of interference from self-pollen deposition is thought to be one reason many self-incompatible taxa possess floral features (such as dichogamy and herkogamy) that are otherwise commonly interpreted as adaptations to promote outcrossing (Lloyd and Webb, 1986; Webb and Lloyd, 1986; Bertin and Newman, 1993; Routley et al., 2004).

In many of the self-incompatible species with self-pollen interference of female function, the self-incompatibility reaction acts late, resulting in ovule discounting under self-pollination (reviewed in Barrett, 2002). An exception is provided by Ockendon and Currah (1977), who studied self-pollen interference in *Brassica oleracea* (kale; Brassicaceae), a species with sporophytic self-incompatibility and stigmatic rejection of self-pollen. In their study, self-pollen reduced the number of outcross pollen tubes by an average of 66% when the outcross-pollen was applied from zero to 16 h following

self-pollination. The authors predicted that self-pollen would also interfere with *B. oleracea* seed production, but they did not test this prediction.

Our goal in this study was to investigate the effect of self-pollen interference on seed set for wild radish, *Raphanus raphanistrum* L., which is a sporophytically self-incompatible, herkogamous species in the mustard family (Brassicaceae). Species in the Brassicaceae include important seed crops as well as species, such as wild radish, that have been used by evolutionary ecologists as model organisms for studying pollinator-based selection on floral morphology and patterns of non-random mating (e.g., Stanton et al., 1986, 1989; Conner et al., 1996; Conner, 1997; Marshall, 1998; Marshall et al., 2000; Mazer and Meade, 2000). In the closely related *R. sativus*, applying pollen from compatible donors in mixed (vs. single) loads affected both the timing of pollen germination and the proportion of seeds sired by the pollen donors (Marshall et al., 1996), but to our knowledge interference has not been examined using combinations of compatible and incompatible (or self-) pollen. We performed controlled pollinations and determined seed set for wild radish plants grown in the greenhouse, using pollen mixtures that represented either self- and outcross-pollen or outcross-pollen alone. We also compared stigmatic pollen loads resulting from our pollinations with those from natural pollinations of the same species to gauge their ecological appropriateness. Our experiment allowed us to answer the following question: does the application of self-pollen along with outcross-pollen significantly reduce seed set in *Raphanus raphanistrum*?

MATERIALS AND METHODS

Study species—*Raphanus raphanistrum* (Brassicaceae) is an herbaceous, annual weed that grows primarily in disturbed habitats, such as abandoned agricultural sites. A native of Europe, the species is widespread across North America. *Raphanus raphanistrum* flowers from May to October. In the United States, peak flowering occurs during the summer months. Its pollinators include

cabbage butterflies, honey bees, and a number of small bee and syrphid fly species (Conner and Rush, 1996). The plants used in our study were grown from *R. raphanistrum* seed collected in a field near Binghamton, New York, USA by J. K. Conner in 1989 (Conner and Via, 1993).

Self-incompatibility in the genus *Raphanus* is sporophytic and controlled by multiple alleles at the *S*-locus (Sampson, 1957, 1967). The *S*-locus alleles display a range of dominance phenotypes in both their stigmatic and pollen incompatibility reactions (Sampson, 1967). Through crossing studies and computer simulation, Sampson (1967) estimated that 25–34 alleles segregated at the *S*-locus for *R. raphanistrum*. Karron et al. (1990) observed cross-incompatibility in 11 to 15% of the within-population crosses with plants from two populations of the closely related *R. sativus*. Self-pollen is less likely to germinate on the stigmatic surface, and the growth of any self pollen-tubes that do form is typically arrested at or near the stigmatic papillae (Dickinson and Lewis, 1973). Although interference competition at the stigmatic surface was observed in *R. sativus* following mixed pollinations with compatible pollen donors (Marshall et al., 1996), rejection of incompatible pollen by stigmatic papilla does not interfere with successful development of adjacent compatible pollen in the closely related genus *Brassica* (Dickinson, 1995). Plants from our study population of *R. raphanistrum* set autonomous seed only rarely (J. K. Conner, Michigan State University, personal observation).

Cultivation and crossing methods—In March 2001, seeds were removed from siliques, and three sibs were planted per pot in 760-cm³ pots filled with Sunshine #4 soil mix (Sun Gro Horticulture, Vancouver, Canada). The pots were placed on benches in the Reed College greenhouse under supplemental lighting provided by high-intensity, sodium-vapor lamps. The plants received daily watering and weekly fertilizing (Peter's Peat-Lite 15-16-17; 200 mg/kg nitrogen; Scotts Co., Marysville, Ohio, USA). Germination began approximately 10 d after sowing. Following germination, seedlings were thinned to one per pot. Pollinations were carried out 3 mo after planting.

Crosses were conducted in two temporal groups (separated by 1 wk) to make the emasculations and pollinations manageable. In total, 34 plants were used, with each serving as both maternal recipient and paternal donor for pollinations. The 34 plants represented 21 field-collected maternal sibships; within each temporal group, all plants came from different maternal sibships ($N = 20$ first group, $N = 14$ second group). Within each temporal group, plants were first randomly paired to create pollen donor pairs. Two pollination treatments were performed with each pollen mixture from a pollen donor pair: the "self/cross" treatment involved pollination of flowers on each of the two donors, while the "cross" treatment involved pollination of flowers on both plants from another, randomly selected pair. Each plant pair received cross-pollen from one of the other pairs. This design ensured that donor number was the same (two) for both pollination treatments. This design also allowed treatment results to be compared using either the pollen donor pairs (paternal, $N = 17$) or pollen recipients (maternal, $N = 34$) as the units of observation, so that we could distinguish the relative paternal and maternal contributions to the observed variation in reproductive success.

To create pollen mixtures, two open flowers from each plant of a donor pair were removed and all 12 anthers placed in a petri dish (24 anthers total). The pollen was then thoroughly mixed together and applied to recipient stigmas with a piece of 23-gauge copper wire. One end of the wire was bent 0.5 cm from the tip, and the bent portion drawn multiple times through the pollen mixture and then drawn across the stigmatic surface of a recipient flower until the pollen was removed.

Crosses were conducted in sets of three flowers on each recipient plant. On the day before pollination, sets of three buds were emasculated before anther dehiscence by removing all six anthers with fine forceps, and the bud was then marked and randomly assigned a treatment (self/cross, cross, or emasculation control). The order in which pollen from each pollen mixture was applied to flowers on the four recipient plants (the donor pair receiving the self/cross treatment and the recipient pair receiving cross treatment) was completely randomized. Between five and nine sets of pollinations were performed on each maternal plant (mean = 6.2 pollinations per recipient plant; variation resulted from different numbers of available buds). Once mature, fruit were collected and placed in a 38°C drying oven, after which the seed number per fruit was determined. The mean number of seeds for each pollination treatment on each maternal plant was used as the dependent variable for analyses, with plant as the unit of observation.

Stigma collection, slide preparation, and pollen counting—For all plants, stigmas were collected from approximately one-third of the flowers (chosen randomly) in each of the two pollination treatments and the emasculation

TABLE 1. Effects of pollination treatment on fruit and seed production and stigmatic pollen load in *Raphanus raphanistrum*.

Treatment	Flowers treated	Fruit no.	Mean seeds per pollination	Mean stigmatic pollen load
emasculation control	211	6	0.08 (0.038)	186.8 (84.4)
cross	212	206	5.09 (0.133)	668.5 (69.1)
self/cross	212	207	5.13 (0.140)	741.1 (62.7)

Note: Values in parentheses are SE. Sample sizes for stigmatic pollen load means: $N = 23$ for self/cross and cross treatments, $N = 16$ for control.

controls on the day following pollination. Collection took place in the Reed College greenhouse. The stigma and as little stylar tissue as possible were removed using a pair of fine forceps, placed on a small amount of basic fuchsin jelly on a slide, and covered with a cover slip (Kearns and Inouye, 1993). After all stigmas for a given day were collected, the slides were taken into the lab, heated to melt the jelly, and compressed to squash the stigmas and spread the pollen grains. Pollen grains on each slide were counted twice using a compound microscope at 100× magnification. On 11 d of the experiment, "ambient" pollen slides were also collected; these consisted of a slide left uncovered during collection of the treatment slides and then covered at the end of collection. This was done to estimate contamination of treatment slides with ambient greenhouse pollen.

Statistical analysis—Results from all 34 plants were combined into a single analysis. A contingency test was used to determine whether the probability of fruit set was independent of pollination treatment (including the emasculation controls). Fruit-set probabilities of self/cross vs. cross treatments were compared using a Fisher's exact test. Seed set was analyzed using means for recipient plants (maternal, $N = 34$) with a paired t test and a null-hypothesis of no difference between self/cross and cross treatments. To determine whether differences among maternal plants or paternal pollen donors contributed significantly to variation in seed set, separate two-way ANOVAs were completed with fruit as the unit of observation and pollination treatment and parent (either maternal recipients or paternal pollen donor pairs) as the independent variables. A power analysis was performed for our paired t test to determine the minimum difference in seed set that would have resulted in statistical significance (Bausell and Li, 2002); a power $(1 - \beta)$ of 0.99 and α of 0.05 were used. A paired t test was used to determine whether stigmatic pollen loads differed between the self/cross and cross treatments, using flowers on donor and nondonor plants treated on the same day with the same pollen mixture.

RESULTS

Fruit set was significantly lower in the emasculation control than in the two pollination treatments ($\chi^2 = 561$, $P < 0.0001$; Table 1). Fewer than 3% of the emasculation controls set fruit, while the pollinations resulted in better than 97% fruit set. Fruit set did not differ between the self/cross and cross treatments (Fisher's exact $P > 0.9999$).

Seed production was nearly identical for the pollination treatments (Table 1; mean maternal difference = 0.02 seeds, one sample $t = 0.155$, $P = 0.88$, $N = 34$). Recipient (maternal) plant explained 53% of the variation in seed number in a two-way ANOVA using fruit as the unit of observation (pollination treatment was the second factor), while pollen donor pair (paternal) explained only 4% of the variation in seed number in a similar two-way ANOVA (pollination treatment was not significant in either analysis).

The standard error for the mean difference in seed production between the pollination treatments (SE = 0.16 for 34 maternal plants) provided sufficient power to give a 99% probability that an observed difference of 0.3 seeds would have been detected as significantly different with a sample size of

30. Our experiment therefore had sufficient power to detect a 6% reduction in seed set in the self/cross treatment. Collection of stigmas 1 d following pollinations did not significantly affect fruit set or number of seeds (data not shown). Pollen loads ranged from 261 to 1513 grains per stigma for the pollinations, with average loads of around 700 grains (Table 1). About 10% more pollen grains were observed on stigmas for the self/cross treatment (Table 1), but this difference was not statistically significant (one sample $t = -0.93$, $P = 0.36$). Pollen loads were significantly smaller for the emasculation controls than for the pollinated treatments (Table 1). For the ambient control slides (on which stigmas were not mounted), pollen counts ranged from 2 to 1470 grains, with a mean of 207 grains and a median of 41 grains. The observation of 1470 pollen grains on a single control slide was most likely due to contamination during stigma collection in the greenhouse where pollen may have been accidentally dislodged from open flowers on a neighboring plant.

DISCUSSION

Our results demonstrate that simultaneous application of self-incompatible pollen does not interfere with seed production from cross-compatible pollen for *R. raphanistrum*. This conclusion is based on our sampling of 21 maternal families from a naturalized population in its introduced range. Our experimental design provided sufficient statistical power that we would have been able to detect a real difference as small as a 6% reduction in seed set. Our method of mixing self- and outcross-pollen before application and then using the same mixture in reciprocal pollinations on both pollen donors ensured that, on average, self-pollen represented 50% of the pollen load in self/cross pollinations. Our design is most similar to pollen deposition from a single pollinator visit that includes some combination of within- and between-flower self-pollen transfer. Although we do not know of any estimates of the ratio of self- and outcross-pollen on stigmas of *R. raphanistrum* in natural populations, this ratio has been reported as approximately 1 : 1 for other species of flowering plants (Thomson and Stratton, 1985; Scribailo and Barrett, 1994). An equal ratio of self- and outcross-pollen is also the most commonly reported treatment used to test for self-pollen interference in other studies (Shore and Barrett, 1984; Bertin and Sullivan, 1988; Galen et al., 1989; Palmer et al., 1989; Broyles and Wyatt, 1993; Scribailo and Barrett, 1994; Nishihiro and Washitani, 1998; Cowan et al., 2000).

The number of pollen grains applied to recipient stigmas was not affected by our pollination treatments. We found no evidence that self-pollen adheres less well than outcross-pollen to stigmatic surfaces for *R. raphanistrum* (as suggested for some mustards; Stead et al., 1979). Dickinson and Lewis (1973) also reported that adherence of self- and outcross-pollen to stigmatic papillae did not differ in the closely related *R. sativus*. Nor did we find evidence that variation in our stigmatic pollen loads contributed to variation in the resulting seed set, which likely indicates we applied a saturating load of pollen on the plants in our study. The average load of c. 700 grains that we observed closely resembles the natural loads we observed in an unpublished field study, with plants grown from seed from the same source population as the current study. In the field study, 72 *R. raphanistrum* were placed into experimental arrays on three separate days in an abandoned field at the Kellogg

Biological Station (Michigan, USA). After each day of exposure to repeated visits from a mix of pollinators (syrphid flies, bees, and lepidopteran species), the mean stigmatic pollen load was 645 grains ($N = 96$ flowers; SE = 38.1; J. K. Conner and K. Karoly, unpublished data). We therefore believe our greenhouse pollinations provided pollen loads representative of those in the field.

Stigmas from our emasculation control flowers had levels of pollen contamination that were significantly lower than in our pollination treatments, but were in the range of stigmatic pollen loads identified as sufficient for seed set in *R. sativus* (Marshall et al., 2000). Because only six of 211 emasculation controls resulted in a fruit, we expect the majority of this pollen contamination was self-pollen (likely originating from un-emasculated, adjacent flowers) or pollen that contaminated the slides during their preparation. Our observation of pollen grains on "ambient" glass slides on which stigmas were not mounted (but which were otherwise treated the same as those slides with stigmas) suggests that extreme care must be taken when preparing slides for pollen enumeration in the vicinity of flowering plants.

Our experimental design allowed us to determine the relative contribution of differences among recipient plants and among pollen donors to the variability in our results. Pollen donor effects deserve consideration if there are pronounced differences among donors in pollen quality (Stone et al., 1995). Our results show, however, that maternal contributions to seed set variability outweigh those of pollen donors, supporting the common practice of using maternal plants as the units of observation for crossing studies.

Our results for *R. sativus* contrast with those of Ockendon and Currah (1977) for *B. oleracea*, which is the only other study we are aware of that examined self-pollen interference for a species in the mustard family. Our two studies differ in several important ways: Ockendon and Currah used recipient plants that were all homozygous for a single *S*-allele at the self-incompatibility locus, they reported stigmatic pollen loads of 2000–3000 grains, and they used sequential application of the two pollen types in their mixed pollinations. The dependent variable (pollen tubes vs. seed set) also differed for our two studies. Given that our two study species are phylogenetically very close relatives (Song et al., 1990; Warwick and Black, 1993, 1997) and similar in both floral morphology and mechanism of self-incompatibility (Ockendon and Gates, 1975; Hodgkin et al., 1998; Hiscock and McInnis, 2003), we believe that the difference in our conclusions results from differences in our methods. We believe that our results reflect the more ecologically relevant test of self-pollen interference for a species in the mustard family because they are based on a diversity of genotypes and reflect pollination conditions similar to those in the field. Our results suggest that self-pollen interference is unlikely to be a mechanism by which natural selection is shaping the evolution of floral morphology for *R. raphanistrum*.

To the best of our knowledge, our study is the first to examine how self-pollen interference affects seed set for a homomorphic species with a sporophytic self-incompatibility system, where rejection occurs at the stigmatic surface. Several of the plant species in which interference has been demonstrated have self-incompatibility mechanisms that act later in development in the ovary (reviewed in Barrett, 2002). To better understand self-pollination interference, research should clarify the relationship between the occurrence of interference and the

mechanism and timing of the self-incompatibility reaction. In addition to further studies with natural populations of mustard species, research with self-incompatible species in the Asteraceae could be especially valuable as that family possesses sporophytic self-incompatibility with stigmatic rejection (Weller et al., 1995). The inflorescence and floral morphologies of Asteraceae species would appear to assure self-pollen deposition, and self-pollen interference is thought to contribute to a decrease in seed set for some Asteraceae (Ehlers, 1999).

LITERATURE CITED

BARRETT, S. C. H. 2002. Sexual interference of the floral kind. *Heredity* 88: 154–159.

BAUSELL, R. B., AND Y.-F. LI. 2002. Power analysis for experimental research: a practical guide for the biological, medical and social sciences. Cambridge University Press, New York, New York, USA.

BERTIN, R. I., AND C. M. NEWMAN. 1993. Dichogamy in angiosperms. *Botanical Review* 59: 112–152.

BERTIN, R. I., AND M. SULLIVAN. 1988. Pollen interference and cryptic self-fertility in *Campsis radicans*. *American Journal of Botany* 75: 1140–1147.

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.

CONNER, J. K. 1997. Floral evolution in wild radish: the roles of pollinators, natural selection, and genetic correlations among traits. *International Journal of Plant Sciences* 158: S108–S120.

CONNER, J. K., AND S. RUSH. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia* 105: 509–516.

CONNER, J. K., S. RUSH, S. KERCHER, AND P. JENNETTEN. 1996. Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). II. Selection through lifetime male and total fitness. *Evolution* 50: 1137–1146.

CONNER, J. K., AND S. VIA. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution* 47: 704–711.

COWAN, A. A., A. H. MARSHALL, AND T. P. T. MICHAELSON-YEATES. 2000. Effect of pollen competition and stigmatic receptivity on seed set in white clover (*Trifolium repens* L.). *Sexual Plant Reproduction* 13: 37–42.

DICKINSON, H. G. 1995. Dry stigmas, water and self-incompatibility in *Brassica*. *Sexual Plant Reproduction* 8: 1–10.

DICKINSON, H. G., AND D. LEWIS. 1973. Cytochemical and ultrastructural differences between intraspecific compatible and incompatible pollinations in *Raphanus*. *Proceedings of the Royal Society of London, B, Biological Sciences* 183: 21–38.

EHLERS, B. K. 1999. Variation in fruit set within and among natural populations of the self-incompatible herb *Centaurea scabiosa* (Asteraceae). *Nordic Journal of Botany* 19: 653–663.

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

HARDER, L. D., AND W. G. WILSON. 1998. A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. *American Naturalist* 152: 684–695.

HISCOCK, S. J., AND S. M. MCINNIS. 2003. Pollen recognition and rejection during the sporophytic self-incompatibility response: *Brassica* and beyond. *Trends in Plant Science* 8: 606–613.

HODGKIN, T., G. D. LYON, AND H. G. DICKINSON. 1988. Recognition in flowering plants: a comparison of the *Brassica* self-incompatibility system and plant–pathogen interactions. *New Phytologist* 110: 557–569.

KARRON, J. D., D. L. MARSHALL, AND D. M. OLIVERAS. 1990. Numbers of sporophytic self-incompatibility alleles in populations of wild radish. *Theoretical and Applied Genetics* 79: 457–460.

KEARNS, C. A., AND D. W. INOUYE. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado, USA.

LLOYD, D. G., AND C. J. WEBB. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. I. Dichogamy. *New Zealand Journal of Botany* 24: 135–162.

MARSHALL, D. L. 1998. Pollen donor performance can be consistent across maternal plants in wild radish (*Raphanus sativus*, Brassicaceae): a necessary condition for the action of sexual selection. *American Journal of Botany* 85: 1389–1397.

MARSHALL, D. L., J. J. AVRITT, M. SHANER, AND R. L. SAUNDERS. 2000. Effects of pollen load size and composition on pollen donor performance in wild radish, *Raphanus sativus* (Brassicaceae). *American Journal of Botany* 87: 1619–1627.

MARSHALL, D. L., M. W. FOLSOM, C. HATFIELD, AND T. BENNETT. 1996. Does interference competition among pollen grains occur in wild radish? *Evolution* 50: 1842–1848.

MAZER, S. J., AND D. E. MEADE. 2000. Geographic variation in flower size in wild radish: the potential role of pollinators in population differentiation. *In* T. A. Mousseau, B. Sinervo, and J. A. Endler [eds.], Adaptive genetic variation in the wild, 157–186. Oxford University Press, New York, New York, USA.

NISHIHIRO, J., AND I. WASHITANI. 1998. Patterns and consequences of self-pollen deposition on stigmas in heterostylous *Persicaria japonica* (Polygonaceae). *American Journal of Botany* 85: 352–359.

OCKENDON, D. J., AND L. CURRAH. 1977. Self-pollen reduces the number of cross-pollen tubes in the styles of *Brassica oleracea* L. *New Phytologist* 78: 675–680.

OCKENDON, D. J., AND P. J. GATES. 1975. Growth of cross- and self-pollen tubes in the styles of *Brassica oleracea*. *New Phytologist* 75: 155–160.

PALMER, M., J. TRAVIS, AND J. ANTONOVICS. 1989. Temporal mechanisms influencing gender expression and pollen flow within a self-incompatible perennial, *Amianthium muscaetoxicum* (Liliaceae). *Oecologia* 78: 231–236.

ROUTLEY, M. B., R. I. BERTIN, AND B. C. HUSBAND. 2004. Correlated evolution of dichogamy and self-incompatibility: a phylogenetic perspective. *International Journal of Plant Sciences* 165: 983–993.

SAMPSON, D. R. 1957. The genetics of self-incompatibility in the radish. *Journal of Heredity* 48: 26–29.

SAMPSON, D. R. 1967. Frequency and distribution of self-incompatibility alleles in *Raphanus raphanistrum*. *Genetics* 56: 241–251.

SCRIBAILO, R. W., AND S. C. H. BARRETT. 1994. Effects of prior self-pollination on outcrossed seed set in tristylous *Pontederia sagittata* (Pontederiaceae). *Sexual Plant Reproduction* 7: 273–281.

SHORE, J. S., AND S. C. H. BARRETT. 1984. The effect of pollination intensity and incompatible pollen on seed set in *Turnera ulmifolia* (Turneraceae). *Canadian Journal of Botany* 62: 1298–1303.

SONG, K., T. C. OSBORN, AND P. H. WILLIAMS. 1990. *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 3. Genome relationships in *Brassica* and related genera and the origin of *B. oleracea* and *B. rapa* (syn. *campestris*). *Theoretical and Applied Genetics* 79: 497–506.

STANTON, M. L., A. A. SNOW, AND S. N. HANDEL. 1986. Floral evolution: attractiveness to pollinators increases male fitness. *Science* 232: 1625–1627.

STANTON, M. L., A. A. SNOW, S. N. HANDEL, AND J. BERECZKY. 1989. The impact of a flower-color polymorphism on mating patterns in experimental populations of wild radish (*Raphanus raphanistrum* L.). *Evolution* 43: 335–346.

STEAD, A. D., I. N. ROBERTS, AND H. G. DICKINSON. 1979. Pollen–pistil interaction in *Brassica oleracea*. *Planta* 146: 211–216.

STONE, J. L., J. D. THOMSON, AND S. J. DENT-ACOSTA. 1995. Assessment of pollen viability in hand-pollination experiments: a review. *American Journal of Botany* 82: 1186–1197.

THOMSON, J. D., AND D. A. STRATTON. 1985. Floral morphology and cross-pollination in *Erythronium grandiflorum* (Liliaceae). *American Journal of Botany* 72: 433–437.

WARWICK, S. I., AND L. D. BLACK. 1993. Molecular relationships in

subtribe Brassicinae (Cruciferae, tribe Brassiceae). *Canadian Journal of Botany* 71: 906–918.

WARWICK, S. I., AND L. D. BLACK. 1997. Phylogenetic implications of chloroplast DNA restriction site variation in subtribes Raphaninae and Cakilinae (Brassicaceae, tribe Brassiceae). *Canadian Journal of Botany* 75: 960–973.

WEBB, C. J., AND D. G. LLOYD. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. II. Herkogamy. *New Zealand Journal of Botany* 24: 163–178.

WELLER, S. G., M. J. DONOGHUE, AND D. CHARLESWORTH. 1995. The evolution of self-incompatibility in flowering plants: a phylogenetic approach. *In* P. C. Hoch and A. G. Stephenson [eds.], Experimental and molecular approaches to plant biosystematics, 355–382. Missouri Botanical Garden Press, St. Louis, Missouri, USA.