Aliso: A Journal of Systematic and Floristic Botany

Volume 21 | Issue 1 Article 2

2002

Genetic Control of Self-Incompatibility in Centromadia (Hemizonia) Pungens Subsp. Laevis (Madiinae, Asteraceae)

Elizabeth A. Friar
Rancho Santa Ana Botanic Garden

Tasha LaDoux
Rancho Santa Ana Botanic Garden

Aliso, 21(1), pp. 1–6

GENETIC CONTROL OF SELF-INCOMPATIBILITY IN *CENTROMADIA (HEMIZONIA) PUNGENS* SUBSP. *LAEVIS* (MADIINAE, ASTERACEAE)

ELIZABETH A. FRIAR AND TASHA LADOUX

Rancho Santa Ana Botanic Garden
1500 N. College Ave.
Claremont, Calif. 91711

ABSTRACT

The presence of self-incompatibility was tested in *Centromadia pungens* subsp. *laevis* and the genetic basis of the self-incompatibility response was explored using crossing studies. We performed full diallel crossing experiments among 10 individuals from one natural population and four F_1 families. We observed a strong self-incompatibility response in all individuals tested, with a significant difference in seed set between selfed and outcrossed matings. Most pairwise matings among parental plants were compatible, with some nonreciprocally incompatible matings (i.e., the matings were successful in one direction, but not the other), and only one reciprocally incompatible mating. The full diallel crossing studies among sibs in the four F_1 families showed two major compatibility classes. These results are consistent with a single-locus sporophytic self-incompatibility system in this species.

Key words: Asteraceae, Madiinae, self-incompatibility.

INTRODUCTION

The majority of flowering plants are hermaphroditic, having both male and female floral organs. The close proximity of the sexual organs allows for self-pollination by pollen transfer within the flower. While self-pollination is common among plants, many mechanisms that promote outcrossing have evolved, implying that there is an evolutionary advantage in avoiding self-fertilization (Richards 1997; Steinbachs and Holsinger 1999). Among the many mechanisms that promote outcrossing, including dioecy, monoecy, distyly, and other morphological adaptations, self-incompatibility (SI) is of interest for several reasons. First, SI is a genetically regulated self-recognition system. Second, the genetic and molecular bases of SI are understood in some, but not all, systems. Third, SI has a wide phylogenetic distribution in the Angiosperms, with several different mechanisms, indicating multiple evolutionary origins (Charlesworth and Charlesworth 1979; de Nettancourt 2001; Lundqvist 1990*a*, *b*; Richards 1997).

Although the phenomenon of SI has been observed for over 200 years (Kölreuter 1763) the genetics controlling it were not discovered until the 1920's (East and Mangelsdorf 1925; Prell 1921) and not until the last 50 years have there been exhaustive pollination studies to elucidate the mechanisms of the different forms of SI. Self-incompatibility systems can be categorized into two basic types, gametophytic SI (GSI) and sporophytic SI (SSI). Gametophytic SI is widespread in the Angiosperms, occurring in at least 15 families (Richards 1997; Newbigin et al. 1994). Crossing success under gametophytic control is determined by the haploid genotype of the individual pollen grain and the diploid genotype of the pistillate parent. Crossing ability in sporophytic systems, on the other hand, is controlled by the diploid genotype of each parent. Thus, a successful cross can only occur when the phenotype, which may be different than the diploid genotype due to dominance interactions between alleles, of each parent is different. Sporophytic SI is known in six plant families, including Asteraceae, Betulaceae, Caryophyllaceae, Convolvulaceae, Sterculiaceae, but has been extensively studied at the molecular level in only one family, Brassicaceae (Kao and McCubbin 1996).

The genetic control of the SI response is relatively easy to characterize by crossing studies of full-sib families. In any F_1 family segregating for four alleles, single-locus GSI and SSI can be distinguished by the crossing behavior of the siblings. One key characteristic of GSI systems is the presence of semi-compatible crosses. In GSI, if the parents share one allele, 50% of pollen grains will be capable of effecting pollination. Gametophytic systems are also characterized by the presence of four equally common crossing types among the F_1 individuals and the absence of nonreciprocal crosses (Goodwillie 1997; Richards 1997). In SSI systems, there are no semi-compatible crosses, but there may be two, three or four crossing types among F_1 individuals, due to dominance relationships among alleles. Also, nonreciprocal crosses are common in SSI sibships, because of dominance relationships among alleles (Richards 1997).

Several crossing studies within Asteraceae support the presence of single-locus SSI, the first studies being of *Cosmos bipinnatus* (Crowe 1954), *Crepis foetida* (Hughes and Babcock 1950), and *Parthenium argen-*

tatum (Gerstel 1950). Additional studies within the family include: *Hymenoxys acaulis* var. *glabra* (DeMauro 1993), *Aster furcatus* (Les et al. 1991), *Senecio squalidus* (Hiscock 2000*a*), *Calotis cuneifolia* (Davidson and Stace 1986), *Helianthus spp.* (Desrochers and Rieseberg 1998), and *Rutidosis leptorrhynchoides* (Young et al. 2000). However, several recent studies have suggested a breakdown of the SSI system, or the presence of additional loci controlling the response, suggesting that the SSI system within Asteraceae may not be as simple as previously thought (Les et al. 1991; DeMauro 1993; Hiscock 2000*b*). These results serve as reminders that exhaustive studies are necessary in order to get a complete picture of the SI mechanism within any given family. Here we present pollination data for *Centromadia pungens* (Hooker & Arnott) Greene subsp. *laevis* (D. D. Keck) B. G. Baldwin, which will be used as the model system for ongoing molecular and genetic studies of SI in Asteraceae subtribe Madiinae.

MATERIALS AND METHODS

Plant Material

Centromadia pungens subsp. *laevis,* formerly *Hemizonia pungens* (Hooker & Arnott) Greene subsp. *laevis* D. D. Keck, is a small annual plant of the South Coast and Peninsular Ranges of Southern California, typically growing in grassland habitats. The inflorescences measure 4–6 mm and have female ray florets and bisexual (functionally staminate) disk florets.

Parental plants were derived from seed collected from the San Jacinto Valley in Riverside County, California in early July 1996 (Michael Wall 146, RSA). These seeds were placed into long-term storage at the Rancho Santa Ana Botanic Garden for future use in reintroduction and restoration projects (accession #19178). Ten seeds from the RSABG seed storage program were randomly chosen and germinated on agar plates, then transplanted into a sterile potting mixture. Plants were grown in a growth chamber at 14 hour days, with daytime temperatures of 26°C and nighttime temperatures of 12°C. Parental plants were numbered 1–10.

Subsequent generation plants were derived from controlled crosses among the parental plants. The achenes were harvested when fully ripe and stored until use. Achenes were germinated and grown as above. F_1 families were numbered 1–4. Individuals in each F_1 family were identified by letters.

Cross- and Self-Pollinations

Parental generation.—A full diallel crossing study was performed on the ten individuals from the wild population to estimate the number of alleles present in the natural population and generate F_1 families for further study. To ensure control of pollen movement, disk florets were removed from heads to be used as female parents prior to anthesis. All pollinations were performed after all ray florets had opened and the stigmatic lobes had reflexed. At that point, heads of the male parent that were presenting pollen were brushed onto the stigmatic lobes. This procedure was followed for both cross- and self-pollinations. Each cross-pollination was performed at least three times. Each self-pollination was performed at least five times. In addition, two heads per individual were simply bagged to determine the amount of seed set without pollen movement. Heads were removed from the plants after 3–4 weeks and the number of filled ray achenes counted. The percentage of seed set was calculated using the number of ray florets and the number of filled achenes. Filled achenes could be easily distinguished based on their plump appearance and indurate achene wall. Unfilled achenes were thin and papery. Several achenes of each type were dissected to verify the correlation between achene appearance and embryo development.

F_1 *generation.*—Full diallel crossing studies were performed on four full-sib families from the parental generation. The four families were chosen to encompass the range of crossing results from the parental generation. Two families represent offspring from a reciprocally successful mating in each direction (#1: 1♂ × 3♀; #3: 3♂ × 1♀), one family represents an additional reciprocally successful mating (#2: 2♂ × 7♀), and one family represents the offspring from a non-reciprocal mating (#4: 7♂ × 1♀; Fig. 2).

Analysis of Crossing Data

The mean number of filled achenes for self- and outcross pollinations was compared using a one-tailed paired *t*-test as implemented in StatView 5.0.1 (SAS Institute, Cary, NC). The correlation between self and outcrossed seed set and male and female success for individual plants was also calculated using StatView 5.0.1.

The crossing results in the parental generation were also examined to determine the number of *S*-alleles present in the sample. *S*-genotypes were assigned to each individual based on crossing phenotype. It was assumed that individuals showing nonreciprocal incompatibility shared one *S*-allele, that dominance hierarchies between alleles were possible, and that all individuals were heterozygous at the *S*-locus. Alleles were defined as recessive if, when shared, the cross was nonreciprocally incompatible. For instance, the 1 × 7 cross is nonreciprocally compatible (i.e., 1♂ × 7♀ is successful, 1♀ × 7♂ is not). It was assumed, therefore, that these two individuals shared an *S*-allele

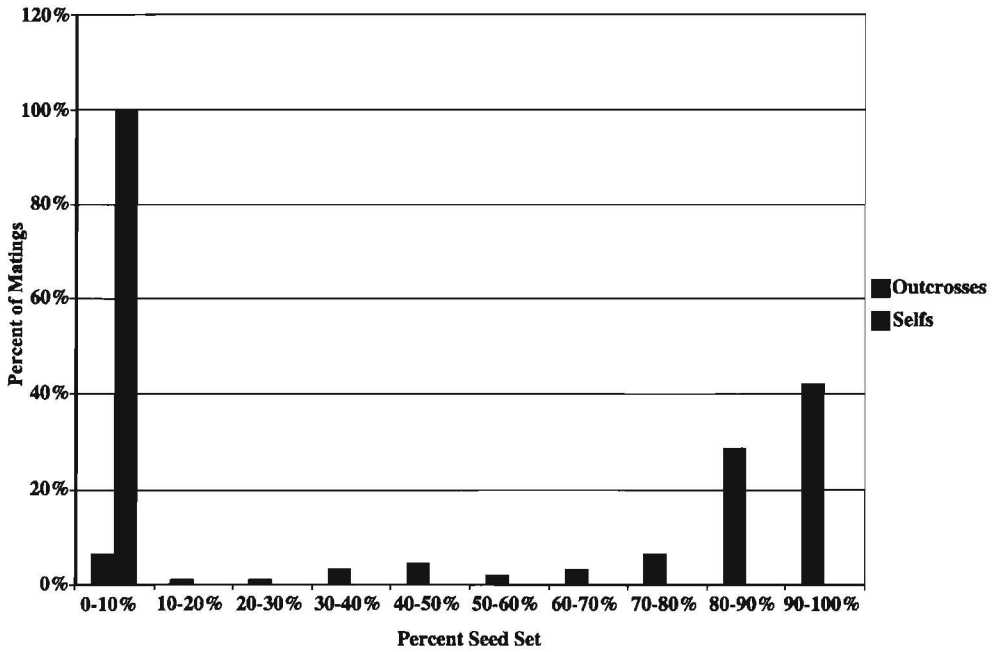

Fig. 1. Percentage of self and outcross matings resulting in various percentages of seed set for the parental generation.

in common. Moreover, as the cross was successful when individual 1 was the male parent, it was assumed that one allele in individual 1 was recessive in the pollen, and that this allele was shared with individual 7. The total number of alleles in the sample was estimated from the *S*-genotype assignments.

RESULTS AND DISCUSSION

Parental Generation

The mean number of ray florets scored per head was 13.8 (4.83 standard deviation), with a mean of 56.6% (40.6%) seed set overall. The mean percentage seed set for outcrossed heads was 65.9% (36.1%). The mean percentage seed set for selfed heads was 0.2% (1.1%). Outcrossing resulted in significantly greater seed set than selfing for each plant (paired *t*-test, $t = -13.126$, $P < 0.0001$). There was no significant correlation between outcross seed set and selfed seed set for individual plants ($r = -0.121$, $P = 0.75$). There was considerable variation in mean seed set (65.1% to 88.04%) and mean male success, defined as mean seed set for an individual over all matings made as a pollen parent (52.7% to 91.2%). However, there was no correlation between success as a male parent and as a female parent for individual plants ($r = 0.203$, $P = 0.59$). Heads that had been bagged without any pollen movement had no observed seed set.

A frequency histogram for the mean percent seed set for both self and outcross matings is shown in Fig. 1. Each bar represents the percentage of matings of each cross type (i.e., self vs. outcross) with the respective percentage of filled achenes. This frequency distribution was used to assign compatibility types for all matings. All matings were categorized as being compatible, incompatible or indeterminate based on the percentage of filled achenes. Incompatible matings were defined as those that had a mean of less than 10% filled achenes, compatible matings were those that had a mean of greater than 40% filled achenes, and indeterminate matings had between 10% and 40% filled achenes. These limits were defined to include the maximum number of outcrosses as compatible and allow for variation in the success of seed set per individual. Only five crosses fell into the 10–40% range; it is unclear whether these represent "leaky" incompatible crosses or compatible crosses with poor seed set. The distribution of matings was highly skewed, with most outcross matings having high percentages of seed set, and all selfed matings and a minority of outcross matings having very low seed set.

Centromadia pungens subsp. *laevis* appears to be strongly self-incompatible. There is a significant difference in the mean seed set between self and outcross matings, with selfed matings resulting in virtually no seed set. Outcross matings resulted in variable amounts of seed set. The distribution of seed set for outcross matings is largely bimodal, with a large peak near 100% and a smaller peak near 0% seed set, with few matings showing intermediate values of seed set. These results suggest that the majority of outcross matings are compatible, with a minority that are strongly incompatible.

Figure 2 shows the distribution of compatible and incompatible matings among the ten individuals from the natural population. Of the 90 possible outcross

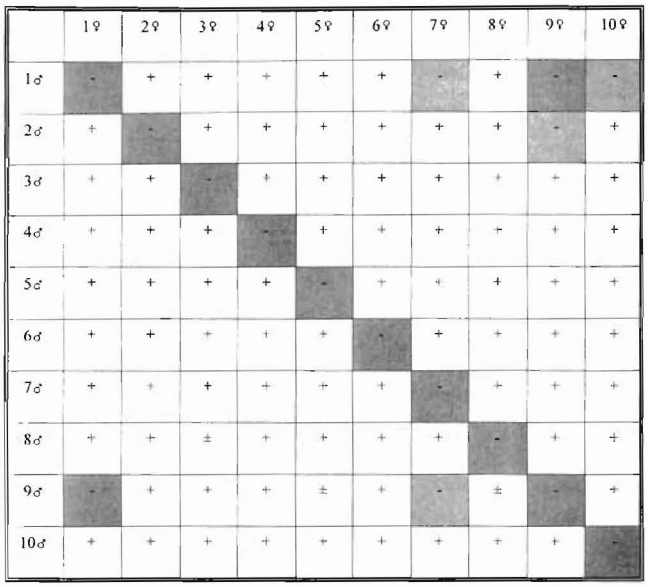

Fig. 2. Diallel crossing results for the parental generation. The male parents are listed in rows, the female parents in columns. A '+' indicates a compatible cross, a '−' an incompatible one, and a '±' an indeterminate one. Light shading indicates nonreciprocally incompatible crosses. Dark shading indicates reciprocally incompatible crosses.

matings, 83 (92.2%) were compatible. Of the incompatible matings, most were nonreciprocally incompatible (i.e., compatible in one direction but not the reverse). Of the 45 pairs of plants, 40 were reciprocally compatible, four were nonreciprocally incompatible, and one was reciprocally incompatible (Fig. 2). The assignment of 16 *S*-alleles to the sample of 10 individuals explains all of the observed mating phenotypes (Table 1). In one incompatible cross (1♂ × 9♀), it is clear that individual 9 shares an allele with individual 2, but which allele cannot be determined, so we arbitrarily assigned allele S_3 to individual 9 for purposes of discussion. In addition, these assignments assume that S_1 is dominant to S_3, but recessive to S_{13}, and S_2 is recessive to S_{16} in pollen in order to explain the nonreciprocal matings.

These allele assignments, while relatively arbitrary, indicate that a sample of ten individuals from the natural population contains approximately 16 *S*-alleles, indicating significant genetic diversity at this locus. These results are typical for self-incompatible taxa, which typically show large numbers of alleles maintained in relatively small populations (Lawrence 2000). By contrast, Brennan et al. (2002) found that a natural population of *Senecio squalidus* had only six alleles in a natural population. This finding was explained by the presence of a severe population bottleneck at the introduction of this species to Britain.

F_1 Generation

Complete diallel crossing experiments were performed with four F_1 families derived from crosses in the parental generation. The four families were chosen to represent the range of crossing results in the parental generation, including both reciprocally compatible and nonreciprocal matings. The sample sizes for the four families vary due to differences in germination rate among them. As the parental generation produced few matings with intermediate percentages of seed set, we were able to score matings in the F_1 generation simply as compatible or incompatible. Therefore, statistics on the percentage of filled seeds were not recorded for the F_1 generation. The results of the diallel crossing experiments are presented in Fig. 3–6.

Table 1. Allelic assignments of individuals in the parental generation based on inference from crossing phenotype.

Individual	Alleles
1	S_1, S_2
2	S_3, S_4
3	S_5, S_6
4	S_7, S_8
5	S_9, S_{10}
6	S_{11}, S_{12}
7	S_1, S_{13}
8	S_{14}, S_{15}
9	S_1, S_3
10	S_2, S_{16}

All families show two major compatibility groups, with occasional aberrant results. Most of the aberrant results appear to be related to low seed set in a few individuals. For instance, in family #2, individuals 2D and 2G never set seed regardless of the male parent used (Fig. 4). Family #4, the only F_1 family resulting from a non-reciprocal cross (Fig. 2; 7♂ × 1♀), showed the most aberrant results. In the inferred allelic compositions of each individual, it was assumed that parental individuals 1 and 7 share an allele in common, either S_1 or S_2, which is recessive in the male parent. The possibility of homozygous individuals resulting from this cross may explain the aberrant results. Alternatively, the aberrant results may be due to poor

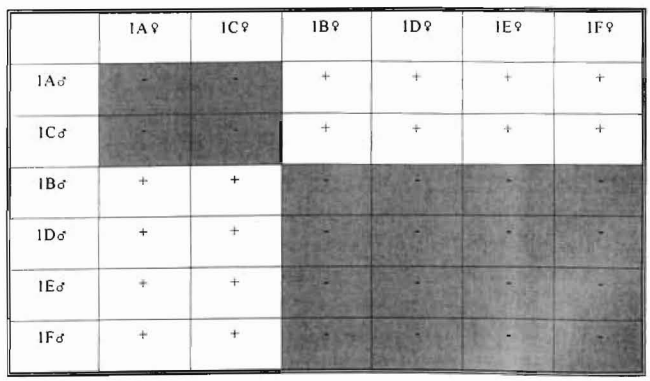

Fig. 3. Diallel crossing results for F_1 family # 1 (1♂ × 3♀). An explanation of symbols and shading is given in Fig. 2.

	2A♀	2D♀	2G♀	2B♀	2C♀	2E♀	2F♀
2A♂	-	-	-	+	+	+	+
2D♂	-	-	-	+	+	+	+
2G♂	-	-	-	+	+	+	+
2B♂	+	-	-	-	-	-	-
2C♂	+	-	-	-	-	-	-
2E♂	+	-	-	-	-	-	-
2F♂	+	-	-	-	-	-	-

Fig. 4. Diallel crossing results for F_1 family #2 (2♂ × 7♀). An explanation of symbols and shading is given in Fig. 2.

male performance for individual 4E and poor female seed set for individual 4D.

The presence of nonreciprocal crossing results in both the parental and F_1 generations and two compatibility classes in the F_1 sibships are consistent with single locus sporophytic control of self-incompatibility. These results are also consistent with previous findings throughout the phylogenetic span of Asteraceae, which have shown single-locus sporophytic control of SI (Gerstel 1950; Hughes and Babcock 1950; Crowe 1954; Hiscock 2000*a*). As SI systems appear to be a family-level trait (with at least one exception in the Polemoniaceae; Levin 1993; Goodwillie 1997; LaDoux and Friar in prep.), all self-incompatible taxa in the Asteraceae may share a similar system (de Nettancourt 2001). However, there was no evidence in these crossing results of the anomalous compatibility found by Hiscock (2000*b*), who found fully or partially compatible crosses in otherwise completely incompatible groups. These results led him to postulate the presence of an additional gametophytic locus controlling the incompatibility response. This pattern has been found in several other self-incompatible species, including *Crepis foetida* and *Hypochoeris radicata* in the Asteraceae, several species in the Brassicaceae, and at least two species in the Caryophyllaceae (Lewis et al. 1988; Lundqvist 1990*a*, 1994; Hiscock 2000*b*).

	3A♀	3D♀	3F♀	3G♀	3H♀	3I♀	3C♀	3E♀
3A♂	-	-	-	-	-	-	+	+
3D♂	-	-	-	-	-	-	+	+
3F♂	-	-	-	-	-	-	+	+
3G♂	-	-	-	-	-	-	+	+
3H♂	-	-	-	-	-	-	+	+
3I♂	-	-	-	-	-	-	+	+
3C♂	-	+	+	+	+	+	-	-
3E♂	-	+	+	+	+	+	-	-

Fig. 5. Diallel crossing results for F_1 family #3 (3♂ × 1♀). An explanation of symbols and shading is given in Fig. 2.

	4A♀	4D♀	4E♀	4H♀	4B♀	4C♀	4F♀	4G♀	4I♀
4A♂	-	-	-	-	+	+	+	+	+
4D♂	-	-	-	-	+	+	+	+	+
4E♂	-	-	-	-	+	-	-	-	-
4H♂	-	-	-	-	+	+	+	+	+
4B♂	+	-	+	+	-	-	-	-	-
4C♂	+	-	+	+	-	-	-	-	-
4F♂	+	-	+	+	-	-	-	-	-
4G♂	+	-	+	+	-	-	-	-	-
4I♂	+	-	+	+	-	-	-	-	-

Fig. 6. Diallel crossing results for F_1 family #4 (7♂ × 1♀). An explanation of symbols and shading is given in Fig. 2.

Recent results (Hiscock et al. 2003) suggest that the self-incompatibility systems of the Asteraceae and Brassicaceae may not be homologous at the molecular level, despite the similarities in physiology and inheritance. The members of the Asteraceae that have been investigated to date seem to share a similar single-locus sporophytic SI syndrome, though with some implications of other modifying loci in addition to the locus of large effect. Once the genes responsible for the SI response in the Asteraceae are cloned, the molecular, developmental, and/or physiological homologies between the two families, and among taxa within the Asteraceae, can be addressed.

ACKNOWLEDGMENTS

The authors wish to thank Eric Roalson and Michael Kinney for help in crossing experiments; Dylan Hannon, Susan Jett and Michael Wall for assistance in seed germination and plant maintenance; and two anonymous reviewers for helpful comments. This work was supported by Rancho Santa Ana Botanic Garden.

LITERATURE CITED

BRENNAN, A. C., S. A. HARRIS, D. A. TABAH, AND S. J. HISCOCK. 2002. The population genetics of sporophytic self-incompatibility in *Senecio squalidus* L. (Asteraceae) I: *S* allele diversity in a natural population. *Heredity* **89**: 430–438.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1979. The evolutionary genetics of sexual systems in flowering plants. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* **205**: 513–530.

CROWE, L. 1954. Incompatibility in *Cosmos bipinnatus*. *Heredity* **8**: 1–11.

DAVIDSON, J. K., AND H. M. STACE. 1986. Genetics of self-incompatibility in *Calotis cuneifolia*. *J. Heredity* **77**: 471–472.

DEMAURO, M. M. 1993. Relationship of breeding system to rarity in the Lakeside Daisy (*Hymenoxys acaulis* var. *glabra*). *Cons. Biol.* **7**: 542–550.

DE NETTANCOURT, D. 2001. Incompatibility and incongruity in wild and cultivated plants. Springer-Verlag, Berlin. 322 p.

DESROCHERS, A. M., AND L. H. RIESEBERG. 1998. Mentor effects in in wild species of *Helianthus* (Asteraceae). *Amer. J. Bot.* **85**: 770–775.

EAST, E. M., AND A. J. MANGELSDORF. 1925. A new interpretation of the hereditary behaviour of self-sterile plants. *Proc. Natl. Acad. Sci. U.S.A.* **11**: 166–171.

GERSTEL, D. U. 1950. Self-incompatibility studies in guayule. *Genetics* **35**: 482–506.

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

HISCOCK, S. J. 2000*a*. Self-incompatibility in *Senecio squalidus* L. (Asteraceae). *Ann. Bot.* **85A**: 181–190.

———. 2000*b*. Genetic control of self-incompatibility in *Senecio squalidus* L. (Asteraceae): a successful colonizing species. *Heredity* **85**: 10–19.

———, S. M. MCINNIS, D. A. TABAH, C. A. HENDERSON, AND A. C. BRENNAN. 2003. Sporophytic self-incompatibility in *Senecio squalidus* L. (Asteraceae)—the search for *S*. *J. Exp. Biol.* **54**: 169–174.

HUGHES, M. B., AND E. B. BABCOCK. 1950. Self-incompatibility in *Crepis foetida* L. subsp. *rhoeadifolia* Bieb. Schinz et Keller. *Genetics* **35**: 570–588.

KAO, T. -H., AND A. MCCUBBIN. 1996. How flowering plants discriminate between self and non-self pollen to prevent inbreeding. *Proc. Natl. Acad. Sci. U.S.A.* **93**: 12059–12065.

KÖLREUTER, J. G. 1763. Vorläufige Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen, nebst Fortsetzungen 1, 2 u. 3, 1761–1766. Ostwald's Klassiker, Nr. 41. Engelmann, Leipzig. 266 p.

LAWRENCE, M. J. 2000. Population genetics of the homomorphic SI polymorphism in flowering plants. *Ann. Bot.* **85A**: 221–226.

LES, D. H., J. A. REINARTZ, AND E. J. ESSELMAN. 1991. Genetic consequences of rarity in *Aster furcatus* (Asteraceae), a threatened self-incompatible plant. *Evolution* **45**: 1641–1650.

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

LEWIS, D., S. C. VERMA, AND M. I. ZUBERI. 1988. Gametophytic-sporophytic incompatibility in the Cruciferae, *Raphanus sativus*. *Heredity* **61**: 355–366.

LUNDQVIST, A. 1990*a*. One-locus sporophytic *S*-gene system with traces of gametophytic pollen control in *Cerastium arvense* ssp. *strictum* (Caryophyllaceae). *Hereditas* **113**: 203–215.

———. 1990*b*. The complex *S*-gene system for control of self-incompatibility in the buttercup genus *Ranunculus*. *Hereditas* **113**: 29–46.

———. 1994. 'Slow' and 'quick' *S*-alleles without dominance interaction in the sporophytic one- locus self-incompatibility system of *Stellaria holostea* (Caryophyllaceae). *Hereditas* **120**: 191–202.

NEWBIGIN, E., M. A. ANDERSON, AND A. E. CLARKE. 1994. Gametophytic self-incompatibility in *Nicotiana alata*, pp. 5–518. *In* E. G. Williams, A. E. Clarke and R. B. Knox [eds.], Genetic control of self-incompatibility and reproductive development in flowering plants. Kluwer Academic, Boston.

PRELL, H. 1921. Das Problem der Unfruchtbarkeit. *Naturwiss. Wochenschr. N. F.* **20**: 440–446.

RICHARDS, A. J. 1997. Plant breeding systems. G. Allen & Unwin, London. 529 p.

STEINBACHS, J. E., AND K. E. HOLSINGER. 1999. Pollen transfer dynamics and the evolution of gametophytic self-incompatibility. *J. Evol. Biol.* **12**: 770–778.

YOUNG A., C. MILLER, E. GREGORY, AND A. LANGSTON. 2000. Sporophytic self-incompatibility in diploid and tetraploid races of *Rutidosis leptorrhynchoides* (Asteraceae). *Austral. J. Bot.* **48**: 667–672.