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TRANSIENT SELF-INCOMPATIBILITY CONFERS DELAYED SELFING IN *LEPTOSIPHON JEPSONII* (POLEMONIACEAE)

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Experimental self- and cross-pollinations revealed that *Leptosiphon jepsonii* (formerly *Linanthus jepsonii*), an annual species, possesses a floral age-dependent form of self-incompatibility (SI) that confers delayed selfing. In field and growth room studies of two populations, self-pollination produced few to no pollen tubes when flowers were first open, but growth of outcross pollen tubes indicated that stigmas were receptive during this phase. By the second day of a flower's duration, however, self pollen tubes grew readily, and pollen tube numbers in self- and outcross-pollinated flowers were not significantly different. Experimental cross-pollinations among full siblings indicated the presence of two mating types, consistent with allelic segregation at a single SI locus. On the basis of these results and the site of pollen rejection, we hypothesize that the floral age-dependent pollen-pistil phenomenon is a modified form of the single-locus sporophytic SI found in a closely related species, *Leptosiphon parviflorus*. To explore the potential effect of transient SI on the timing and rate of self-fertilization, we used allozyme genetic markers to determine the paternity of seeds produced by mixed self and outcross pollen loads. Consistent with the pollen tube data, nearly all progeny from mixed pollinations of day 1 flowers were outcrossed, and the proportion of selfed progeny increased significantly with floral age. Transient SI may be an evolutionarily stable strategy or a transitional step in the evolution from SI to complete selfing.

Keywords: breakdown of self-incompatibility, mixed mating system, delayed selfing, *Leptosiphon jepsonii*, *Linanthus jepsonii*, floral age-dependent self-incompatibility.

Introduction

A continuing focus in the study of plant mating system evolution concerns mixed mating systems, i.e., species in which both self- and cross-fertilization occur at substantial frequencies. Interest has centered in part on a long-standing debate as to the stability of mixed mating systems. Theoretical work by Lande and Schemske (1985), which models the joint evolution of the selfing rate and inbreeding depression, predicts that populations will evolve toward either complete selfing or complete outcrossing; the finding of bimodal selfing rates in a survey of plant species provided support for their model (Schemske and Lande 1985; but see Vogler and Kalisz 2001). The mixed mating systems that do exist may be interpreted as transitional between outcrossing and selfing, yet a number of theoretical studies have identified conditions under which mixed mating is expected to be evolutionarily stable (Holsinger 1986; Uyenoyama 1986; Latta and Ritland 1994; Johnston 1998).

How selection acts on mating systems will be influenced by the mode of self-fertilization, e.g., whether selfing is autonomous or mediated by a pollinator, and by the relative timing of self- and cross-fertilization (Lloyd 1979, 1992; Lloyd and Schoen 1992). For example, Lloyd (1979) argued that the evolution of delayed selfing, in which self-fertilization occurs after opportunities for outcrossing, should have less restrictive selective requirements than other forms of selfing because it assures reproduction but does not preempt cross-fertilization when mates and pollination vectors are available. A variety of mechanisms for delayed selfing have been identified in plant species (reviewed in Kalisz et al. 1999) including temporal changes in stigma-anther position (Kalisz et al. 1999) and self-pollination during corolla abscission (Dole 1990).

Self-incompatibility (SI), a genetic mechanism that inhibits self-fertilization, is a common feature of many angiosperms (de Nettancourt 1977). Of the array of outcrossing features of higher plants, strong SI is perhaps the most effective in promoting obligate cross-fertilization. Yet in a number of studies, SI has been found to be leaky or partially effective because of genetic modifiers (Levin 1996; Hiscock 2000; Good-Avila and Stephenson 2002) or developmental (Ascher and Peloquin 1966; Vogler et al. 1998) or environmental factors (Wilkins and Thorogood 1992; Jakobsen and Martens 1994). Thus, the presence of SI in some species can result in mixed mating.

Here we present an investigation of the mechanisms that promote mixed mating in *Leptosiphon jepsonii* (formerly *Linanthus jepsonii* until recent taxonomic revision [Porter and Johnson 2000]). The genus *Leptosiphon* comprises both self-incompatible and highly selfing species, and phylogenetic reconstruction indicates that SI has been lost multiple times (Goodwillie 1999). The loss of SI has been accompanied in most lineages by marked reduction in flower size (Goodwillie

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1999; Goodwillie and Stiller 2001), suggesting that these self-compatible species are primarily self-fertilizing; however, the large-flowered species *L. jepsonii* is visited by pollinators and is partially outcrossing (Goodwillie 2000). The mating system of *L. jepsonii* varies both spatially and temporally (Goodwillie 2000; D. Schemske, unpublished data), suggesting that the species is a facultative outcrosser in which the rate of selfing depends on the availability of pollinator service.

The observation of intermediate and variable outcrossing rates motivated an investigation of the timing and mechanism of mixed mating in *L. jepsonii*. Preliminary observations uncovered a pollen-pistil phenomenon that may confer delayed selfing in the species. Flowers of *L. jepsonii* initially are fully self-incompatible and then become self-compatible within 24–48 h. Similar phenomena, in which SI becomes less effective as flowers age, have been reported in a number of taxa, including the well-studied perennial *Campanula rapunculoides* (Campanulaceae; Richardson et al. 1990).

To characterize this phenomenon, we carried out a series of experiments (1) to demonstrate that rejection of self pollen is transient in *L. jepsonii*, (2) to explore the potential effect of transient SI on the timing and extent of self-fertilization, and (3) to provide evidence that the transient pollen-pistil phenomenon in *L. jepsonii* is controlled by a single *S*-type locus and is most likely a modified form of the SI system found in closely related species.

Material and Methods

Species and Site

Leptosiphon jepsonii and its highly selfing sister species *Leptosiphon bicolor* comprise a lineage derived from a self-incompatible ancestor (Goodwillie 1999). The distribution of *L. jepsonii* is restricted to Napa and Sonoma Counties in northern California, where populations occupy open grassy areas in oak woodlands (Schemske and Goodwillie 1996). Study populations were located at two sites in Napa County that were separated by several miles: Mast Hill (MH) and the Wantrup Reserve (WR).

The flowering period of *L. jepsonii* extends from early April to late May, and fruits mature ca. 4 wk after flowering. Plants usually have one but may have as many as three head-like inflorescences, each with three to 15 flowers. On any given day, generally one, or occasionally two to three, flowers are open per plant. At ca. 1000 hours, flowers open, and anthers dehisce immediately. Emasculated and unfertilized flowers can last up to 6 d, but flowers senesce ca. 1 d after fertilization occurs. Typical floral longevity in the field is 2–3 d (C. Goodwillie, unpublished data). Populations are visited and pollinated by beeflies (Bombyliids) that collect both pollen and nectar.

In a previous greenhouse study of *L. jepsonii* populations at MH and WR, seed set in hand self-pollinated flowers was not significantly different from that in cross-pollinated flowers (Goodwillie 2000), suggesting complete self-compatibility. In the same experiment, autonomous self-pollination produced more than 96% of the seed set in fruits where self pollen was applied by hand (Goodwillie 2000). The study populations were found to be largely self-fertilizing in 1995; outcrossing rate estimates for the MH and WR populations in 1995 were 0.14 and 0.30, respectively (Goodwillie 2000). For an adjacent population in Napa County, the 1993 outcrossing rate was found to be 0.45 (D. Schemske, unpublished data).

Pollen Tube Growth: Growth Room Experiment

In fall 2002, 32 plants from each population were raised from field-collected seeds. Each seed was taken from a different maternal plant, separated by at least 1 m in the population. Seeds were germinated on moist filter paper at 4°C and then planted in Conetainers (Stuewe and Sons) in standard potting soil. Seedlings were raised in a growth chamber, and mature plants were moved to a growth room with artificial and natural light in which pollination experiments were carried out. Conetainers were subirrigated continuously and watered once weekly with a mild solution of 12-55-6 commercial fertilizer.

To examine temporal changes in pollen-pistil interactions, we quantified pollen tube growth in flowers subjected to four treatments: (1) cross pollen applied on the first day that a flower was open (hereafter, day 1 flowers), (2) self pollen applied on day 1 flowers, (3) self pollen applied on day 2 flowers, and (4) self pollen applied on day 3 flowers. The four pollination treatments were replicated on three flowers on each of the experimental plants from the MH and WR populations. For cross-pollinations, flowers were first emasculated. Anthers of two haphazardly chosen pollen donors from the same population were then brushed across the stigma lobes. For self-pollination treatments on day 2 and day 3, flowers were emasculated on the morning of day 1, and petals were marked with a colored dot on consecutive days to keep track of flower age.

In all of the treatments, pollen from fresh day 1 flowers was used. Thus, while stigma age and pollen source varied with treatment, pollen age was held constant. Preliminary studies indicated that pollen age does not influence pollen-pistil interactions. To further test that hypothesis, we carried out pollinations of day 1 stigmas on 20 plants from the MH population using self pollen from day 1 or day 2 flowers. Each treatment was replicated on two flowers.

In all treatments, pollinations were conducted between 900 and 1030 hours. Three hours after pollen was applied, styles were removed and fixed in 70% ethanol : 30% acetic acid. Styles were transferred to 10 M sodium hydroxide for 4–6 h and then to a solution of 0.1% aniline blue in 33 mM potassium phosphate. Stigmas and styles were inspected for pollen tube growth using epifluorescent microscopy (Martin 1959). We counted all pollen tubes that had grown at least to the base of the stigma. By 3 h after pollination, most pollen tubes had grown well down the >3-cm style. Pollen tubes were counted to a maximum number of 60, above which we found that accuracy in counting diminished rapidly.

Pollen tube data showed substantial departures from normality, in part because of the upper bound imposed by our counting method. For this reason, we analyzed pollen tube data by using nonparametric tests throughout the study. To determine the effect of pollination treatments on pollen tube

number, we calculated individual plant means (of three replicate flowers) for each treatment and applied Friedman rank tests (SPSS 2002), which assume only that experimental plants were mutually independent. Post hoc multiple comparisons were conducted using an analogue of the Bonferroni pairwise comparison procedure (Neter et al. 1990). In each population, we tested for significant variation among experimental plants in the number of self pollen tubes on day 1 by using a Kruskal-Wallis test, with individual flowers as the unit of replication.

Pollen Tube Growth: Field Experiment

Experimental pollinations to assess pollen tube growth in self- and cross-fertilized flowers were replicated in field conditions. In May 2003, one newly opened flower was marked on each of 150 plants at WR, and 50 plants were randomly assigned to each of three treatments: (1) pollen from two outcross donors applied on day 1 flowers, (2) self pollen applied on day 1 flowers, and (3) self pollen applied on day 2 flowers. Pollen was applied as flowers began to open between 1000 and 1100 hours. All experimental plants were enclosed in a single large mesh tent to exclude pollinators. At 1400 hours on the day that pollen was applied, flowers were removed, and styles were fixed. Styles were later cleared and stained, and pollen tubes were counted.

By necessity, the design of the field study differed somewhat from the growth room experiment. We deemed it preferable to carry out all treatments simultaneously to control for day-to-day variation in temperature. Because flower number is limited in field-grown plants (plants often produce as few as four flowers), and only one flower per plant is typically open at a time, a different plant was used for each replicate of each treatment in the field experiment. We could not use day 1 pollen for day 2 selfing treatments, as was done in the growth room study, because it was difficult to find plants with two flowers opening on sequential days. Instead, for day 2 self-pollinations, we left flowers unmanipulated on day 1 and brushed within-flower self pollen on the stigma on day 2. Though spatial overlap of stigma and anthers promotes autonomous self-pollination in older flowers of *L. jepsonii*, day 1 flowers of most plants show a slight initial stigma-anther separation (C. Goodwillie, unpublished data). As a result, self pollen deposition usually did not occur before it was applied by hand on the morning of day 2. Finally, because pollen was generally scarce in day 3 flowers, we did not include that treatment in our field experiment.

The effect of treatments on pollen tube growth was examined using a Kruskal-Wallis test (SPSS 2002), which assumes only that plants were sampled randomly for each treatment and that plants used for each of the three treatments were independent. Post hoc comparisons were conducted to test for significant differences between treatment pairs by using an analogue to the Bonferroni pairwise comparison procedure (Neter et al. 1990).

Siring Success of Self and Cross Pollen

To determine the potential effect of transient SI on the timing and rate of self-fertilization, we used allozyme genetic markers to identify the paternity of seeds produced when mixed pollen loads were applied. In *L. jepsonii* populations, two allelic variants at a 6-phosphogluconate dehydrogenase (6-PGD) locus can be distinguished using allozyme electrophoresis. In a preliminary study, a 6-PGD heterozygote was self-fertilized. The frequencies of progeny genotypes were consistent with the 1 : 2 : 1 genotype ratios expected with Mendelian segregation (56 homozygous fast : 111 heterozygous : 56 homozygous slow, $\chi^2 = 0.134$, $P > 0.9$), which indicates that the 6-PGD locus can be considered a neutral marker for the purposes of this study.

Six pairs of plants were selected for use in the experiment, each comprising one fast and one slow 6-PGD homozygote. Mixed pollen loads were applied by hand to the stigmas of emasculated flowers on both plants of each pair. A comparable pollen load was gathered from each parent, and the pollen was mixed and applied to stigmas with a fine paintbrush. Applications of mixed pollen were carried out on ca. 20 day 1 and 20 day 2 flowers of each plant, and in some plants on 20 day 3 flowers as well. Calyces of pollinated flowers were marked with colored ink to indicate flower age at pollination, and seeds were collected from mature fruits. Seeds from each maternal plant and treatment type were pooled for genotyping. For example, all seeds from the 20 day 1 pollinated flowers on plant 1 were combined, and from these, 60 seeds were genotyped. In some cases, fewer than 60 seeds were available (mean = 50.28). By genotyping seeds before germination, we minimized the potential confounding factor of inbreeding depression, at least that occurring after seed maturation. Proteins were extracted in a sucrose buffer (0.1 M Tris base, 0.5 M sucrose, 0.02 M sodium bisulfite, pH 7.5; K. Ritland, personal communication) from seeds that had been hydrated for at least 3 h. Potato starch gels (11%) were electrophoresed using a morpholine citrate buffer system. Stain and buffer recipes and electrophoresis protocols followed those of Werth (1985).

For each parent plant and floral age treatment, progeny were tested for deviation from the null expectation of 1 : 1 selfed to outcrossed progeny. The proportion of outcrossed progeny derived from pollinations of day1 versus day 2 and day 2 versus day 3 flowers were compared using paired *t*-tests (SPSS 2002). Because progenies of paired plants were not independent, we used the mean of paired plants as our unit of replication for this test.

Genetic Control of Transient SI

We hypothesized that the transient pollen-pistil interaction observed in pilot experiments is a modified form of the single locus SI system present in *L. parviflorus*, a closely related species. To test that hypothesis, we carried out a crossing experiment with 16 full siblings. If pollen-pistil interactions are controlled by a single *S* locus, we should find evidence for segregation of four or fewer distinct *S* genotypes in the progeny array.

Two unrelated individuals were grown in the growth room to serve as the maternal and paternal plant for the sibling array, and self-pollinations of day 1 flowers were carried out to verify that both were transiently self-incompatible. Flowers for cross-pollination were emasculated as buds to prevent self-pollination. Seeds were collected from mature fruits, and

16 sibling plants were grown in the growth room. Siblings were crossed in a full diallel design, and each of the 264 crosses was replicated on three day 1 flowers. In a subset of six sibling plants, experimental pollinations also were carried out on day 3 flowers in a full diallel design. Pollinations, staining, and pollen tube counts were carried out as described above for the growth room experiment.

Results

Pollen Tube Growth: Growth Room Experiment

Pollen tube data showed dramatic temporal changes in the stigma affecting SI function. In an overall Friedman test, pollen tube number was significantly different among outcross pollinations and self-pollinations of day 1, 2, and 3 flowers in both populations (MH: $\chi^2 = 60.32$, df = 3, $P < 0.0001$, $n = 31$; WR: $\chi^2 = 63.48$, df = 3, $P < 0.000$, $n = 32$; fig. 1). Abundant pollen tube growth was observed in day 1 flowers that were cross-pollinated (population means = 55.32 for MH, 46.90 for WR), indicating that stigmas are receptive as soon as the flowers open. In contrast, very few self pollen tubes were observed in day 1 flowers (population means = 8.51 for MH, 5.26 for WR). Pollen tube number for self and cross pollen treatments on day 1 differed significantly in post hoc pairwise tests for both populations. Individual means for self pollen tube numbers on day 1 were greater than 10 in only four plants in MH and five plants in WR. We observed a dramatic increase in self pollen tubes from day 1 to day 2 flowers (population means for day 2 = 44.31 for MH, 45.00 for WR), and this difference was significant in pairwise tests for both populations. In fact, pollen tube number for self-pollination on day 2 did not differ significantly from that of cross-pollination on day 1 in either population. In post hoc tests for both WR and MH, self pollen tube numbers on day 3 (population means = 52.46 for MH, 51.49 for WR) were not significantly different from those on day 2. Mann-Whitney tests showed no significant difference between the two populations in the pollen tube numbers for any of the self-pollination treatments (day 1: $U = 405$, $P = 0.19$; day 2: $U = 398$, $P = 0.16$; day 3: $U = 410$, $P = 0.17$). Comparison of self pollen tube number when day 1 and day 2 pollen was applied to day 1 stigmas found no effect of pollen age on SI (mean for day 1 pollen = 0.8, day 2 pollen = 0.9, $Z = -0.447$, $P = 0.655$).

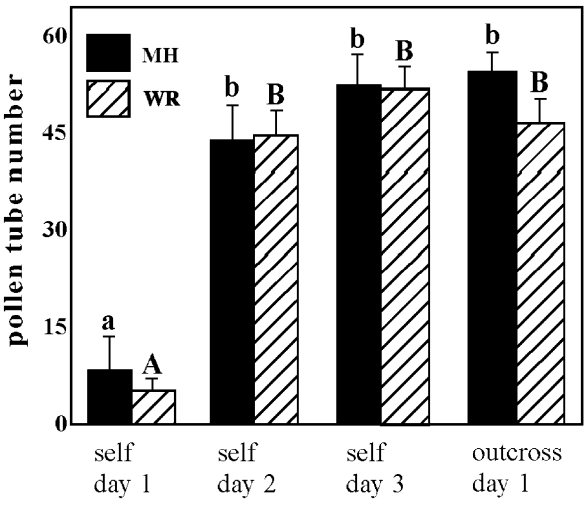

Fig. 1 Pollen tube numbers for self-pollination of day 1, day 2, and day 3 flowers and outcross-pollination of day 1 flowers. Treatments were replicated on 31 plants from Mast Hill (*MH*) and 32 plants from Wantrup Reserve (*WR*). Bars represent standard errors. Letters above bars indicate treatment means that were not significantly different in post hoc tests carried out for each population.

Despite an overall trend toward transient SI, we observed substantial and significant variation among individuals in the number of self pollen tubes produced in day 1 flowers (MH: $\chi^2 = 75.2$, df = 30, $P < 0.0001$; WR: $\chi^2 = 76.7$, df = 31, $P < 0.0001$; fig. 2). While the population mean for self pollen tubes in day 1 flowers was <9 in both populations, individual plant means ranged from 0 to 45 tubes in MH and from 0 to 60 tubes in the WR population.

Pollen Tube Growth: Field Experiment

The field experiment at WR yielded similar results to the growth room experiment and provided further evidence for transient SI in *Leptosiphon jepsonii*. Pollen tube number differed significantly among pollination treatments ($\chi^2 = 50.38$, df = 2, $P < 0.0001$, n for each treatment ranged from 48 to 50). Post hoc pairwise comparisons showed that pollen tube numbers for day 2 selfing and day 1 outcrossing were not significantly different, but pollen tubes with day 1 selfing were significantly lower than either of the other treatments. The mean for selfing on day 1 flowers (5.67 pollen tubes) was very similar to that in the growth room experiment (5.26), although the means for day 2 selfing (22.37 pollen tubes) and day 1 outcrossing (30.54) were somewhat lower than those seen in the growth room (55.32 and 46.90, respectively). As in the growth room experiment, we observed substantial variation among field plants for pollen tube number in day 1 flowers. Values ranged from 0 to 60, and the percent of individuals that produced more than 10 pollen tubes with day 1 selfing (14.6%) was comparable to that seen in the growth room study (15.6%).

Siring Success of Self and Cross Pollen

When mixed self and outcross pollen loads were applied to the stigmas of day 1 flowers, the proportion of outcrossed progeny (as indicated by heterozygosity at the 6-PGD locus) approached 1 (mean = 0.97), indicating that SI is highly effective when flowers first open (table 1). The proportion of seeds that were sired by outcross pollen on day 2 flowers (mean = 0.875) was significantly lower ($t = 2.613$, df = 5, $P = 0.047$), reflecting a shift toward self-compatibility as flowers age. No significant difference in the proportion of outcrossed progeny was observed between day 3 (mean = 0.717) and day 2 flowers ($t = 1.949$, $P = 0.123$). Despite an increase after day 1 in self pollen siring success, progeny of day 2 and 3 flowers of nearly all of the plants showed a significant excess of outcrossed progeny relative to the null expectation of equal frequencies (table 1). In

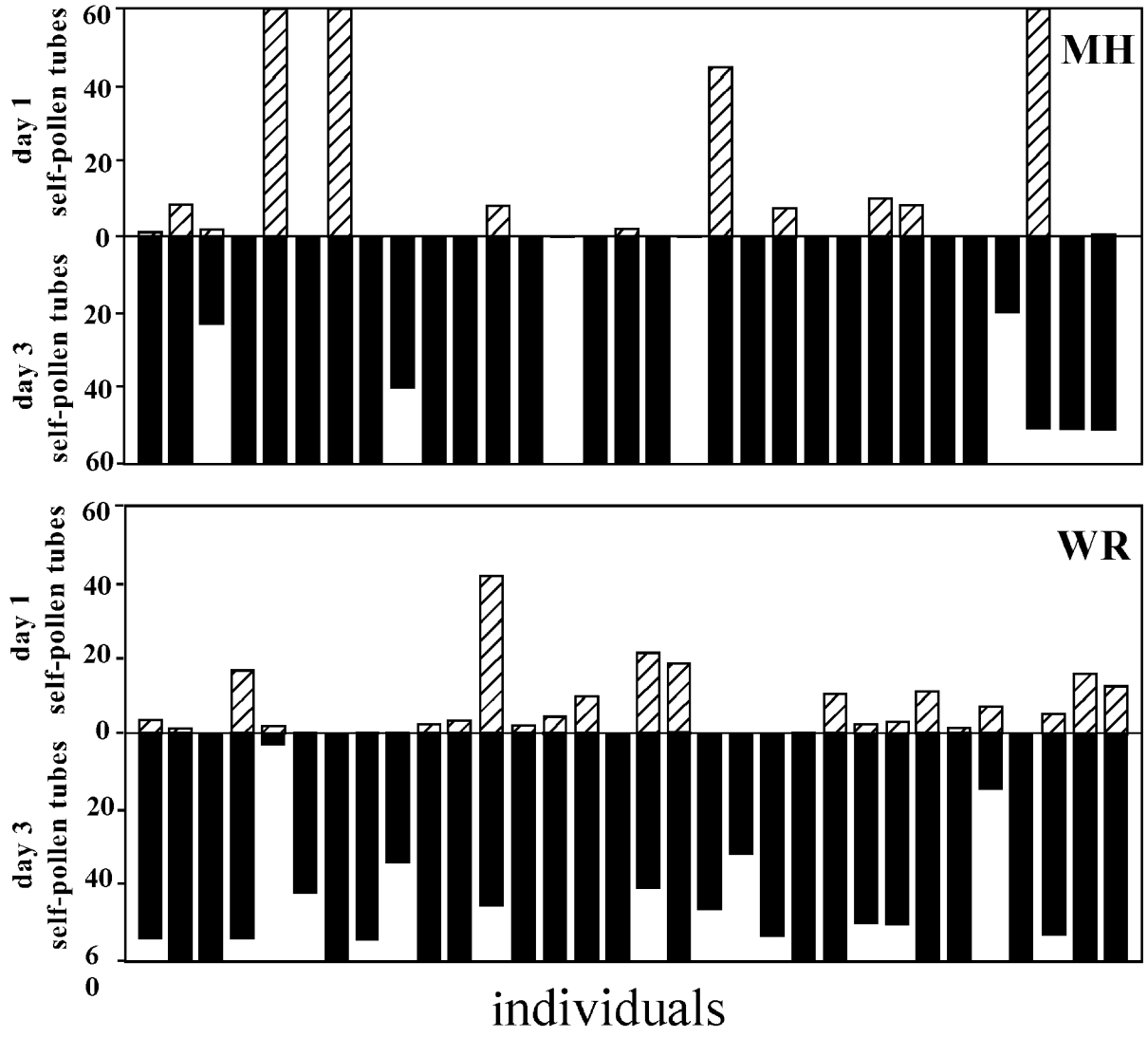

Fig. 2 Individual means (of three replicate flowers) for day 1 and day 3 self-pollinations on plants from Mast Hill (*MH*) and Wantrup Reserve (*WR*).

only one progeny set, day 3 flowers on plant 7, were there significantly fewer outcrossed progeny than expected under the null hypothesis.

Genetic Control of Pollen-Pistil Interactions

Results of cross-pollinations in day 1 flowers indicated the presence of two mating types in the full sibling array (fig. 3). The mean number of pollen tubes in all day 1 crosses fell into two distinct classes: <3, in which case the crossed plants were considered to be cross-incompatible, or >40, in which case siblings were deemed to be cross-compatible. For a small number of crosses, compatibility could not be assessed because flowers were not available (fig. 3). In a subset of the siblings comprising six plants of the same mating type, pollinations carried out on day 3 flowers yielded a mean of more than 30 pollen tubes in all crosses, confirming that expression of incompatibility among siblings is dependent on floral age.

Discussion

Pollen tube observations in field and growth room experiments showed that *Leptosiphon jepsonii* populations exhibit a transient form of SI in which flowers of most individuals switch from fully self-incompatible to self-compatible during the first 24 h of anthesis. Although stigmas are fully receptive to outcross pollen when flowers first open, selfing produces few pollen tubes. Thus, young flowers generally exhibit classic and highly effective SI. By the second day of a flower's duration, however, self pollen tubes grow readily, with pollen tube number approaching or equal to that in outcross-pollinated flowers. Our experiments indicate that the transformation in SI occurs in the stigma.

Table 1

Proportion of Outcrossed Progeny (as Determined by Genotype at the 6-PGD Locus) Derived from Application of 50% Self and 50% Outcross Pollen Loads on Flowers of Different Ages

	Age of flowers at time of pollination		
Plant no.	Day 1	Day 2	Day 3
1	1.000 (61)	0.968 (62)	...
2	1.000 (61)	0.885 (61)	0.706 (51)
3	1.000 (59)	1.000 (59)	...
4	0.989 (91)	0.817 (82)	**0.536** (82)
5	1.000 (61)	0.742 (62)	...
6	0.950 (60)	0.815 (65)	...
7	0.704 (27)	0.750 (36)	0.23 (39)
8	1.000 (60)	0.986 (69)	...
9	1.000 (32)	0.957 (23)	0.909 (55)
10	1.000 (62)	0.629 (70)	0.871 (62)
11	1.000 (55)	1.000 (78)	1.000 (28)
12	1.000 (55)	0.927 (55)	0.757 (37)
Means	0.97051 (684)	0.875 (722)	0.717 (354)

Note. Sample size of progeny array shown in parentheses. Paired plants served as reciprocal outcross pollen donors for mixed pollinations. Proportions that did not deviate significantly from the null expectation (0.5) are shown in bold. Paired plants are 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, and 11 and 12.

The pollen-pistil phenomenon observed in *L. jepsonii* is mostly likely a modified form of the SI system characterized in a congeneric and closely related species, *Leptosiphon parviflorus* (Goodwillie 1997). First, the physiological mechanism appears to be the same in the two species, with rejection of self pollen grains occurring at the stigmatic surface. Second, the finding of two distinct mating types segregating in a sibship array for *L. jepsonii* indicates the presence of a single-locus system, as was found in *L. parviflorus*. Data from crossing experiments for multiple sibships in *L. parviflorus* supported a hypothesis of sporophytic SI (Goodwillie 1997). Incompatibility relationships in the single *L. jepsonii* sibship reported here do not provide enough information to distinguish between gametophytic and sporophytic control of SI. For instance, a sibship comprising only two mating types could result from either type of system when parent plants share one *S* allele. Given the similarity in the site of rejection and close evolutionary relationship between the species, however, our preliminary conclusion is that the transient SI mechanism in *L. jepsonii* is sporophytically based and is homologous to the fully effective system found in congeners.

Congruent with the data for pollen tube observations, the siring success of self pollen grains increased with flower age, as evidenced by a significant increase in the proportion of selfed progeny produced by mixed self and outcross pollen loads. However, we observed fewer selfed progeny in older flowers than we expected on the basis of the abundant self pollen tube growth in day 2 flowers. We offer several possible explanations. First, although germination and pollen tube initiation of self and outcross pollen may be comparable in older flowers, the rates of pollen tube growth may differ and lend a strong siring advantage to outcross pollen in mixed loads. This phenomenon has been observed in other species (Bateman 1956; Cruzan and Barrett 1993; Eckert and Allen 1997). Second, environmental factors such as temperature or developmental factors such as the age, health, or prior fruit set have been shown to influence the strength and timing of SI (Wilkins and Thorogood 1992; Jakobsen and Martens 1994; Vogler et al. 1998) and may have affected our results. Unfortunately, we do not have complete day 1 and day 2 pollen tube count data for the plants used in the genetic marker study. However, incomplete data for some of the experimental plants indicated lower numbers of self pollen tubes in day 2 flowers than was observed in the pollen tube study. Finally, although a previous study found relatively low inbreeding depression in *L. jepsonii* (Goodwillie 2000), the possibility exists that inbreeding depression may have resulted in the abortion of selfed seeds before maturation. Although we used seed tissue for genotyping to eliminate the confounding factor of inbreeding depression expressed in germination or growth, we could not control for early seed abortion.

We observed significant variation within both populations of *L. jepsonii* in the degree and duration of SI. Although the majority of individuals were transiently self-incompatible, several plants in each population were fully self-compatible at anthesis, and two plants in each population produced fewer than 15 self pollen tubes in day 3 flowers. In follow-up experiments with two of the latter class of plants, we confirmed that the self pollen was viable when used for cross-pollinations and found no seed set with self-pollination. Thus, it appears that individuals in these populations range from fully self-incompatible to fully self-compatible. Because plants that differed in SI were raised in fairly uniform environmental conditions in the growth room, it seems likely that

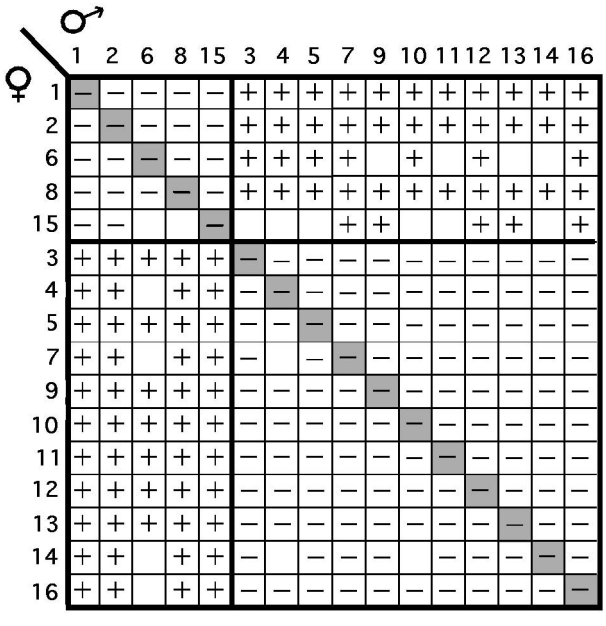

Fig. 3 Results of sibling crosses on day 1 flowers. A plus sign denotes a compatible cross (mean pollen tube number > 40); a minus sign denotes an incompatible cross (mean pollen tube number < 5). Numbered siblings have been rearranged according to inferred mating type. Empty cells indicate missing data.

the variation is genetically based. Moreover, the distribution of self pollen tube numbers in day 1 flowers, particularly in the MH population, indicates that the variation may have a simple genetic basis; with polygenic inheritance, we would expect to find more intermediate phenotypes. Experimental crosses are currently under way to characterize the basis of this variation.

The results of our pollen tube observations and paternity study indicate that pollen-pistil interactions in *L. jepsonii* promote delayed selfing. The high percentage of outcrossed progeny from mixed pollen loads, even in day 2 and 3 flowers, indicates that the potential for outcrossing is considerable. Nevertheless, field outcrossing rates for the two populations in 1995 were quite low, indicating that pollinator visitation was limited in that season. It appears that delayed self-compatibility confers reproductive assurance in *L. jepsonii*. A 2-yr pollen limitation study showed that reproduction was generally less pollen limited in populations of *L. jepsonii* than in populations of *L. parviflorus*, a closely related, fully self-incompatible species (Goodwillie 2001). Future studies will test explicitly for reproductive assurance by comparing seed set in emasculated versus open pollinated flowers in natural populations.

The transient pollen-pistil interaction in *L. jepsonii* bears a resemblance to phenomena that have been reported in a handful of other species (Ascher and Peloquin 1966; Stephenson et al. 1992). It is worth noting as well that similar phenomena might easily have been overlooked in other plant taxa. For instance, in the case of *L. jepsonii*, determination of SI based on seed set in selfed and crossed flowers (a commonly used protocol) would indicate complete self-compatibility. In the most thoroughly studied example of floral age-dependent SI, individuals of *Campanula rapunculoides* (Campanulaceae) have been shown to have higher seed set from self-pollination in day 4 relative to day 1 flowers (Stephenson et al. 1992), and mixed self and outcross pollen loads produce a higher proportion of selfed offspring in older flowers (Vogler and Stephenson 2001). Stephenson et al. (2000) suggest that floral age-dependent SI in *C. rapunculoides* may allow it to persist when pollinator service is low, populations are small, or *S* allele diversity is limited. Despite overall functional similarity, it seems likely that different mechanisms may underlie floral age-dependent SI in some of the plant taxa for which it has been reported. While variable and often subtle increases in self-compatibility with floral age have been observed in *C. rapunculoides* (Good-Avila and Stephenson 2002), the transition to self-compatibility observed in flowers of *L. jepsonii* appears to be quite sudden and complete, indicating a different physiological process at work.

The phenotypically plastic and genetically variable expression of SI observed in *C. rapunculoides* combined with similar reports in other taxa have prompted Stephenson et al. (2000) to make the case that SI acts less often as a qualitative mechanism for obligate outcrossing than as a quantitative phenomenon, conferring a flexible mating system that responds to environmental and developmental factors. *Leptosiphon jepsonii* provides another example in which the mating system appears to be influenced by both the presence of a modified form of SI and the availability of pollinators and mates. The evolutionary implications of partial SI are still largely unknown, however. Partial SI can be interpreted in a number of ways: as a mechanism maintained by selection to provide flexibility in the mating system (Stephenson et al. 2000), as a transitional step toward complete loss of SI and a high selfing rate, or, in some cases, perhaps as an incidental by-product of an imperfection in the SI mechanism that has little selective consequence. For most taxa in which partial SI has been reported, we have little information that allows us to distinguish between these alternative hypotheses. In *L. jepsonii*, the presence of transient stigma-anther separation accompanying transient SI would appear, at face value, to indicate the coadaptation of developmental, morphological, and physiological mechanisms to promote delayed selfing. However, temporal changes in stigma-anther separation also are seen in *L. parviflorus*, a closely related and fully SI species, which suggests that the phenomenon is a preexisting condition rather than an adaptation for delayed selfing. Nevertheless, it may have played a role in the evolution of transient SI or may presently serve some function in the timing of self-fertilization.

In *Leptosiphon*, highly selfing and highly outcrossing mating systems appear to be more common than the intermediate condition seen in *L. jepsonii*. Several congeneric species have complete SI in at least parts of their range. For instance, in a survey of several populations of *L. parviflorus*, SI was found to be fully effective even in older flowers, and no variation in the strength of SI was detected among individuals or populations (C. Goodwillie, unpublished data). Efforts to breach SI by any of the means used in other taxa (bud pollinations, high temperatures, CO_2, etc.) have been unsuccessful (C. Goodwillie, unpublished data; D. Schemske, personal communication). At the other extreme, preliminary investigations of 10 populations of *Leptosiphon bicolor*, the small-flowered sister taxon to *L. jepsonii*, indicate that the species is fully self-compatible on anthesis (C. Goodwillie, unpublished data), and mating system estimates indicate nearly complete selfing (Goodwillie 2000). *Leptosiphon bicolor* and *L. parviflorus* are abundant and geographically widespread; both are found throughout the California floristic province, and *L. bicolor* extends further north to Washington state. Thus, it seems that the mating system extremes represented by these two taxa are highly successful in the genus. Despite the potential flexibility offered by delayed selfing, *L. jepsonii* is relatively uncommon and is restricted to Napa and Sonoma Counties. Transient SI may be a recently evolved, highly successful, and evolutionarily stable state, destined perhaps for range expansion. Alternatively, *L. jepsonii* may represent a transitional step in the breakdown of SI, on a trajectory toward complete or nearly complete selfing.

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