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# TIMING OF SELF-COMPATIBILITY, FLOWER LONGEVITY, AND POTENTIAL FOR MALE OUTCROSS SUCCESS IN *LEPTOSIPHON JEPSONII* (POLEMONIACEAE)[1]

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When fertilization triggers flower senescence, early autonomous selfing may cause flowers to senesce before pollen has dispersed, discounting unused pollen. Selfing-induced flower senescence was examined in *Leptosiphon jepsonii*, a species that varies in the timing of self-compatibility. In field and greenhouse experiments, fertilization had a large effect on flower senescence; most outcrossed flowers senesced after 1 d whereas emasculated flowers lasted 2–5 d. In a comparison of inbred lines from three populations, longevity of autonomously selfed flowers of early self-compatible individuals was significantly less than that of late self-compatible individuals. In field experiments, autonomously selfed flowers were shorter-lived in a predominantly early-selfing population than in a predominantly late-selfing population. Pollen was available and viable beyond the first day of anthesis, suggesting that reductions in flower longevity caused by autonomous selfing could incur a cost to male outcross fitness. We argue that this effect is likely to be most pronounced under intermediate rates of pollinator visitation. Observed pollinator visitation rates ranged from 0.035–0.775 visits per flower per day, indicating a potential for selfing-induced flower senescence to incur pollen discounting in *Leptosiphon jepsonii*.

**Key words:** flower senescence; mating system; Polemoniaceae; pollen discounting.

The evolution of flower longevity has been hypothesized to involve a tradeoff between male- and female-fitness accrual rates and the costs of floral maintenance or construction (Primack, 1985; Ashman and Schoen, 1994, 1997; Schoen and Ashman, 1995). In many angiosperms, flower senescence is triggered by fertilization (Gori, 1983; Stead, 1992; Yasaka et al., 1998; Underwood et al., 2005), a process mediated by ethylene in some species (Stead, 1992; O'Neill, 1997; van Doorn, 2002; Rogers, 2006). From an evolutionary perspective, flower longevity in these species is hypothesized to be shortened after fertilization to minimize the resource expense and water loss of maintaining open flowers (Stead, 1992; Ashman and Schoen, 1994).

The effect of self-fertilization (selfing) on flower senescence has received little attention (Sato, 2002). In particular, the selective role of this effect in evolution of the selfing rate has rarely been considered. Fertilization-induced flower senescence has potential consequences for mating system evolution because autonomous selfing (selfing that occurs without a pollinating vector) decouples the rates of fitness accrual through ovule fertilization and pollen dispersal. When fertilization is entirely pollinator-mediated, as in an obligately outcrossing hermaphrodite species, both male and female fitness depend on the action of the same pollen vector. Therefore, fitness through ovule fertilization and pollen dispersal must be correlated to some extent, although they may accrue at different rates (Ashman and Schoen, 1994; Schoen and Ashman, 1995). Thus, in an outcrossing species, ovule fertilization is expected to trigger flower senescence only after substantial pollen has been dispersed. In contrast, autonomous self-fertilization of ovules that occurs early in anthesis may trigger flower senescence before any opportunities for dispersal of pollen by a vector. As such, selfing-induced flower senescence might be viewed as a potential source of pollen discounting, which is defined as a reduction in male outcross success that results from self-fertilization (Holsinger et al., 1984).

Pollen discounting plays an important role in mating system evolution (Feldman and Christiansen, 1984; Holsinger, 1991) because it erodes the genetic advantage of selfing proposed by Fisher (1941). Fisher's principle states that, while outcrossing individuals on average transmit one copy of their genes as mother to their own seeds, selfing individuals will be mother and father to their own seeds as well as fathering seeds of other individuals in the population. This confers to selfing individuals a 50% advantage in gene transmission and leads to the prediction that self-fertilization will evolve when inbreeding depression is less than 0.5 (Maynard Smith, 1977; Lloyd, 1979; Charlesworth, 1980; Lande and Schemske, 1985). Pollen discounting has been defined and modeled as the extent to which pollen grains used for self-fertilization reduce the pollen pool available for outcross siring events (see Harder and Wilson, 1998 for a review of definitions). In the context of selfing-induced flower senescence, we use the term in a more general sense to refer to any reduction in male outcross success associated with self-fertilization (Holsinger et al., 1984).

The consequences of selfing-induced flower senescence will depend on the pollination context. Pollen dispersal is minimal when visitation is low, so floral longevity has little consequence for male fitness. With high pollinator visitation rates, opportunities for pollen dispersal are likely to precede flower senescence. At intermediate visitation rates, however, selfing-induced flower senescence may limit opportunities for male success through pollen dispersal.

Here we quantify selfing-induced flower senescence in an annual plant, *Leptosiphon jepsonii*, and suggest a relationship between this phenomenon and male outcross success. Individuals of *L. jepsonii* vary in the timing of self-fertilization. A

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floral age-dependent form of self-incompatibility prevents early self-fertilization in some individuals, conferring delayed selfing, while others are self-compatible upon flower opening (Goodwillie et al., 2004). The frequency of early self-compatible individuals varies significantly among populations and is strongly associated with the population outcrossing rate (Goodwillie and Ness, 2005). Variation in the mode of selfing within and among populations motivated us to explore the evolutionary forces that play a role in this system. Delayed selfing is expected to be advantageous because it avoids costs of inbreeding depression, which in populations of *L. jepsonii* are low but not negligible (Goodwillie, 2000; Goodwillie and Knight, 2006). Conversely, early selfing may provide more effective reproductive assurance when pollinators are absent. The current study addresses yet another potential selective consequence of this variation.

We address several questions concerning flower longevity and self-fertilization in *Leptosiphon jepsonii*: Does the timing of fertilization affect the rate of flower senescence? If autonomous selfing limits flower longevity, flowers are expected to be shorter lived in early self-compatible plants than in transiently self-incompatible plants. What is the condition of pollen throughout anthesis? For selfing-induced senescence to incur pollen discounting, pollen must be viable and available for dissemination beyond the first day of anthesis. Finally, what do pollinator visitation rates for *L. jepsonii* suggest about flower longevity and its effect on male outcross success?

## MATERIALS AND METHODS

***Biology of Leptosiphon jepsonii***—The study species, *Leptosiphon jepsonii* (D.W. Schemske & C. Goodwillie) J.M. Porter & L.A. Johnson (formerly *Linanthus jepsonii*) of the family Polemoniaceae, is a small annual restricted to the California North Coast Range (Schemske and Goodwillie, 1996). The flowering season extends from April to late May. In the field, plants typically have one inflorescence with 1–3 flowers open at a time.

*Leptosiphon jepsonii* is transiently self-incompatible. In field and growth-room studies, self-pollination of some individuals produced few to no pollen tubes when flowers first opened, but by the second day of anthesis, self pollen tubes grew readily. In contrast, some individuals in populations of *L. jepsonii* are capable of producing self-pollen tubes at the start of anthesis (Goodwillie et al., 2004). The establishment of self-fertilized lines that are true-breeding for early self-compatibility or transient self-incompatibility indicates that this variation is genetically based (C. Goodwillie, unpublished data).

***Pollination protocol***—Pollination experiments were designed to test the hypothesis that fertilization triggers flower senescence and, more specifically, that the timing of self-fertilization affects the rate of flower senescence. In both growth-room and field experiments, four treatments were applied to newly opened flowers: (1) hand-outcrossed, (2) unmanipulated, (3) hand-selfed, and (4) emasculated. Hand-outcrossed flowers were emasculated in the bud to prevent self-pollen deposition. Flowers were observed at noon each day after treatments were applied, and the longevity of each flower was recorded in increments of days. A flower was considered senesced when petals were closed or the flower was no longer upright and therefore unavailable to pollinators. A comparison of outcrossed and emasculated flowers tested the hypothesis that fertilization triggers flower senescence. Unmanipulated flowers senesce under autonomous self-fertilization. The hand-selfed treatment controlled for factors that prevent self-pollen deposition, such as stigma–anther separation.

***Growth-room experiment***—For the growth-room experiment, self-fertilized lines that were bred for either early self-compatibility ("early SC") or transient self-incompatibility ("late SC") were used. The experiment was carried out on three pairs of early and late SC lines, each pair derived from seeds of different field populations located in Santa Rosa, Lake Hennessey, and Calistoga, California, USA and subjected to six generations of self-fertilization.

TABLE 1. Mean outcrossing rates and mean number of days a flower is self-incompatible (SI) for LH, WR, and IC populations of *Leptosiphon jepsonii*.

| Mating system parameter | LH | WR | IC |
|---|---|---|---|
| Mean population outcrossing rate ($t$) | 0.06 | 0.37 | 0.69 |
| Mean number of days a flower is SI | 0.4 | 0.9 | 1.6 |

*Note*: Outcrossing rates represent the mean of 2 (LH and IC) or 3 (WR) years. All data are from Goodwillie (2000) and Goodwillie and Ness (2005). Mean number of days was calculated from the frequency of individuals that are SI for 0, 1 or 2 days. LH = Lake Hennessey; WR = Wantrup Reserve; IC = Ida Clayton Road.

The pollination treatments described were replicated on eight individuals of each line. A set of all four treatments was applied to flowers of an individual on the same day and repeated five times per individual.

To test hypotheses concerning the effects of fertilization on flower longevity, we carried out nested analysis of variance with number of days as the dependent variable. Population (seed source of the inbred lines) was included in the initial analysis, with SC type nested within population. Because no significant differences were detected among populations, data from the three sets of lines were pooled, yielding a model with pollination treatment and SC type (both fixed factors) and plants (random factor) nested within SC type. Pairwise treatment comparisons were assessed with Games–Howell tests for data with unequal variances. These data did not meet the assumption of homogeneity of variances, and transformations did not improve the fit, but the complexity of our experimental design precluded using a nonparametric alternative. Analysis of variance has been shown to be fairly robust to these violations (Lindman, 1992). Moreover, individual nonparametric Kruskal–Wallis tests for each inbred line yielded qualitatively congruent results concerning significant differences between pollination treatments in flower longevity (results not shown). Analyses were done with the program SPSS (SPSS, 2004).

In addition we tested our major hypothesis that, under autonomous selfing, flower longevity is less in early SC than in late SC plants. For each individual, a composite variable was created by dividing the mean longevity of unmanipulated flowers by the mean for emasculated flowers, which gives the proportion of maximum flower longevity that occurs with autonomous selfing. Data were pooled across populations, and a $t$-test (equal variances not assumed) was carried out to examine differences between SC types in the composite variable.

***Field experiments***—Ideally, field experiments would replicate growth-room experiments to test differences in flower senescence between early and late SC variants within populations. In the field, however, the phenotype of individual plants cannot be assessed readily. As a complementary approach, we carried out the experiments in three populations that contrast in the frequency of early and late SC individuals (Table 1); the populations were located at Lake Hennessey (LH), Wantrup Reserve (WR), and Ida Clayton Road (IC).

Pollinations took place under a tent made of tulle (pore size of 1 $mm^2$) to exclude pollinators. Because plants produce a very limited number of flowers, the four treatments could not be replicated on the same individuals. Instead treatments were replicated on a single flower of 30 individuals randomly distributed within each experimental site, with care taken to ensure that treatments were not repeated on the same individual.

To test hypotheses concerning the general effects of pollination on flower senescence, we carried out a two-way analysis of variance with pollination treatment and population as fixed factors. Populations were considered a fixed factor because they were chosen specifically to represent contrasting frequencies of early and late SC (Table 1). Tukey tests were used to test the significance of pairwise comparisons for pollination treatments. Analyses were done with the program SPSS (SPSS, 2004).

***Pollen availability and viability***—We compared pollen availability on the first and second day of anthesis. At each field site, dehisced anthers were collected from 60 individuals, from 30 flowers on the first day and 30 on the second of anthesis. Pollen collections were made as close to morning anthesis as possible. Because these plants were not enclosed by netting, reduced pollen counts on the second day might reflect pollen exported by pollinators in addition to that removed by wind or gravity (see Results for pollinator observations). Thus, pollen counts on the second day should be considered a

TABLE 2. Results from the growth-room experiment. Nested analysis of variance for the effect of pollination treatment on flower longevity in early SC and late SC lines of *Leptosiphon jepsonii*.

| Source of variation | df | MS | *F* |
|---|---|---|---|
| Pollination treatment | 3 | 168.030 | 277.76*** |
| Error | 143 | 0.605 | |
| SC type | 1 | 50.777 | 11.93** |
| Error | 47 | 4.257 | |
| Individual (SC type) | 47 | 4.324 | 7.09*** |
| Error | 141 | 0.610 | |
| Pollination treatment × SC type | 3 | 5.400 | 8.93*** |
| Error | 143 | 0.605 | |
| Pollination treatment × Individual (SC type) | 141 | 0.610 | 1.98*** |
| Error | 768 | 0.307 | |

*Note*: SC = self-compatible; MS = mean square. ** $P \leq 0.01$; *** $P \leq 0.001$.

minimum estimate of the amount available in flowers that have not been visited. Anthers were stored in microcentrifuge tubes of lactophenol blue solution (containing aniline blue, which dyes pollen). Pollen was counted from two samples per flower using a hemacytometer.

We used a pollen tube growth assay to compare pollen viability of flowers on the first and second day of anthesis. One-d-old and 2-d-old anthers were used to pollinate stigmas on day-one flowers of a different individual. Each treatment was replicated on two flowers of 13 individuals. Stigmas were collected, fixed in 3 : 1 ethanol-acetic acid, cleared in 10 N sodium hydroxide, and stained in 0.1% aniline blue in 33 mM potassium phosphate. Pollen tubes were observed and counted using an epifluorescent microscope.

***Pollinator visitation and observation***—Pollinator observations were carried out in 1 × 3 m areas for 1 h and repeated eight times at each of the three field sites. Both effective and ineffective pollinator visits were observed, and the number of effective visits per pollinator was recorded during each session. A visit was considered effective if contact was made between the reproductive organs of the flower and the insect. Flower densities were recorded for each sampling area. In previous field studies, we observed that pollinators of *L. jepsonii* were generally active between 1000 and 1700 hours (C. Goodwillie, personal observation). From these data, the mean number of pollinator visits per flower per day was calculated for each site.

## RESULTS

***Growth-room experiment***—Analysis of variance revealed that all main effects (treatment, SC type, and plants) significantly affected flower longevity (Table 2, Fig. 1). Pairwise comparisons for treatments showed that outcrossed flowers were significantly shorter lived than emasculated flowers, indicating that fertilization triggers senescence in *L. jepsonii*. Unmanipulated flowers were significantly longer-lived than hand-selfed flowers, which suggests that self-fertilization is limited in part by lack of self-pollen deposition. Of particular interest are differences in flower longevity between SC types. The interaction of SC type and pollination treatment was significant, and inspection of means indicated that the largest differences between early and late SC lines were in unmanipulated and hand-selfed treatments (Fig. 1). Unmanipulated flowers of late SC individuals lasted a mean of 2.23 d in comparison to 1.43 d in early SC individuals. Congruently, unmanipulated flowers of early SC individuals had a significantly smaller proportion of maximum longevity (0.484) than those of late SC individuals (0.689, $t = 5.046$, $df = 35.7$, $P < 0.001$). This indicates that when flowers self-fertilize autonomously, early SC limits flower longevity.

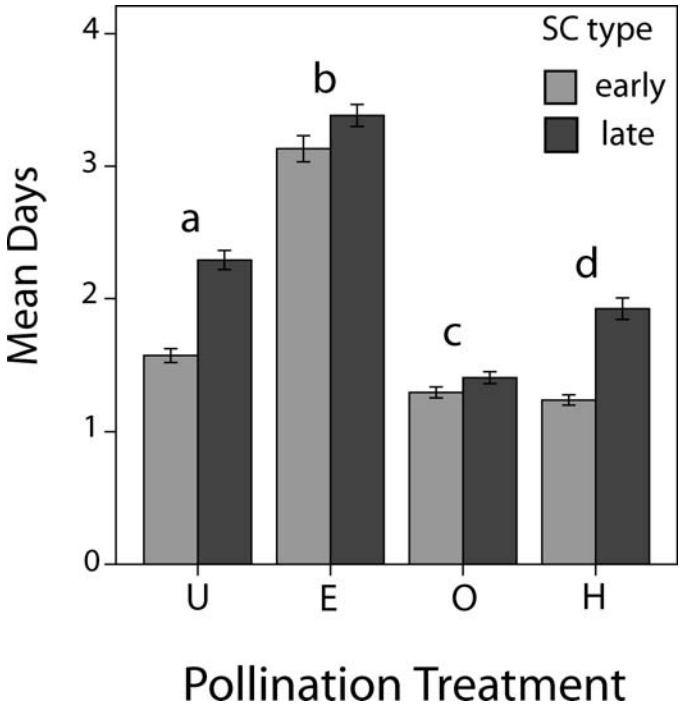


Fig. 1. Results from growth-room experiment. Mean flower longevity for four pollination treatments (U, unmanipulated; E, emasculated; O, hand-outcrossed; and H, hand-selfed) in early and late self-compatible (SC) inbred lines of *Leptosiphon jepsonii*. Data from three sets of lines are pooled. Letters above bars indicate treatments that differed significantly in post-hoc tests. Error bars represent ±1.00 SE.

***Field experiment***—Flower longevity was affected significantly by pollination treatments ($F_{3,384} = 64.06$, $P < 0.001$, Fig. 2), with all pairwise comparisons significantly different at $P < 0.001$ in post-hoc tests. Congruent with the growth-room results, outcrossed flowers were significantly shorter-lived than emasculated flowers. Hand-selfed flowers were significantly shorter-lived than unmanipulated flowers, indicating that lack of autonomous self-pollen deposition limits self-fertilization. This difference was most pronounced in the IC population (Fig. 2), which is consistent with the observation that flowers in that population have greater stigma–anther separation than those at WR or LH (Goodwillie and Ness, 2005). Significant variation in flower longevity was found among populations ($F_{2,384} = 7.488$, $P < 0.01$). A significant interaction between population and pollination treatment ($F_{6,384} = 5.930$, $P < 0.001$) supports the hypothesis that flower longevity is limited by early SC. Inspection of population means reveals that mean flower longevity varies most among populations in unmanipulated flowers (Fig. 2). Moreover, the trend for mean longevity in unmanipulated flowers is consistent with our expectations based upon the frequencies of early SC in each population (Table 1, Fig. 2).

***Pollen availability and viability***—A considerable portion of day-1 pollen was still available on the second day of anthesis at each site (86% of 1367, 36% of 2958, and 53% of 2272 for LH, WR, and IC, respectively). Pollen tube growth assays showed substantial pollen tubes (more than 60 for every sample) from both 1-d-old and 2-d-old pollen. These data indicate that pollen

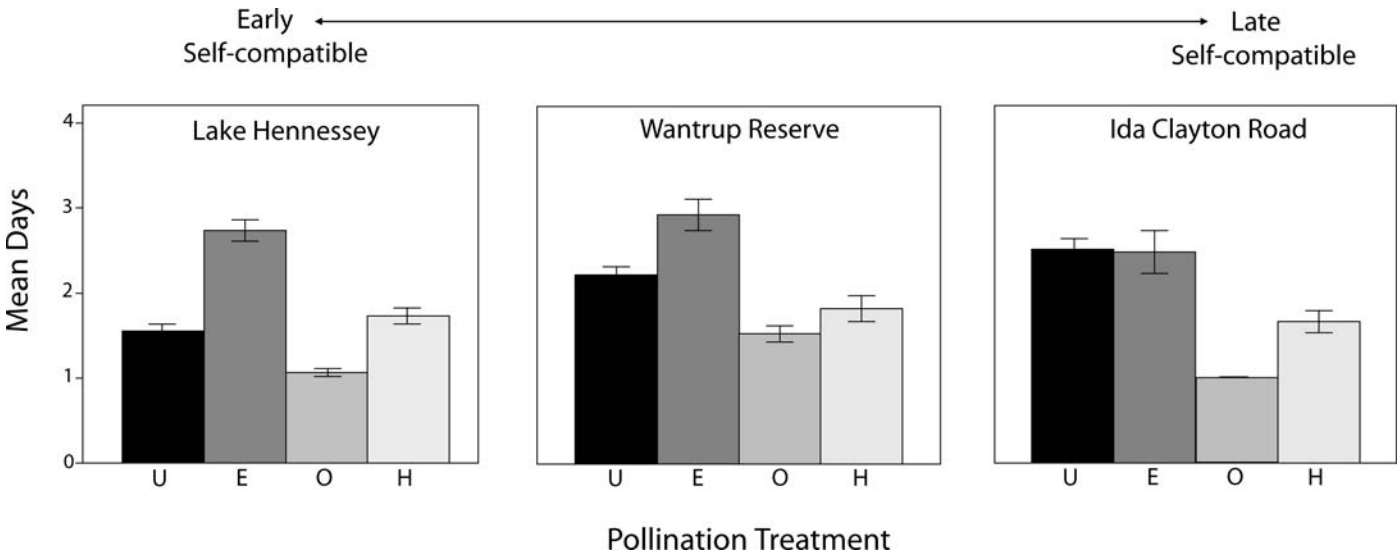


Fig. 2. Results from field experiment. Mean flower longevity for four pollination treatments in three populations of *Leptosiphon jepsonii*. Treatment abbreviations are the same as those in Fig. 1. Error bars represent ±1.00 SE.

is not only available but viable and a potential cost to male outcross success if discounted early in anthesis.

***Pollinator visitation and observation***—The mean number of pollinator visits per flower per day was 0.035, 0.28, and 0.78 for LH, WR, and IC, respectively. Visitation rates at each site increased with the known outcrossing rate (Table 1). Visits per flower per day at each site were less than 1.00, indicating that not every flower is visited on every day. Beeflies (Bombyliidae; order, Diptera) and bees (order, Hymenoptera) were observed as effective pollinators, but the proportion of visits by these insects varied among field sites. At both LH and IC, the majority of visits were by beeflies (77% and 96%, respectively), whereas at WR only 31% of visits were by beeflies and the remainder by bees. In addition three ineffective pollinator visits by butterflies (Pieridae, order Lepidoptera) were observed at IC.

## DISCUSSION

Selfing limits flower longevity in *Leptosiphon jepsonii* through fertilization-induced senescence. Experiments showed that, without manipulation and with the exclusion of pollinators, the timing of self-compatibility significantly affected the rate of flower senescence. For instance, our growth-room results showed that autonomous selfing triggers senescence faster in early SC flowers than in late SC flowers. In the field, unmanipulated flowers of the predominantly early SC population (LH) had an average longevity of 1.55 d, as compared to the predominantly late SC population (IC) with an average of 2.51 d. The potential for increased male outcross success in longer-lived flowers was supported by our finding of substantial availability of viable pollen beyond the first day of anthesis.

The potential strength of senescence-related pollen discounting is expected to depend on the pollinator context. At low pollinator visitation rates, pollen discounting is always negligible because pollen used for self-fertilization has minimal outcrossing potential. At high pollinator visitation rates, selfing-induced senescence is not a likely source of pollen discounting. In this scenario, high levels of cross-fertilization result in the export of pollen before senescence. With intermediate visitation rates, however, the expanded window of opportunity for pollen dispersal may increase outcross male fitness. The LH population had very low visitation rates, suggesting that outcrossing potential is low and that pollen discounting is negligible at this site. The WR and IC populations experienced much higher pollinator visitations rates. Despite these higher visitation rates, the average number of pollinator visits per flower per day was less than one at both sites, and longer-lived flowers are expected to have higher male outcross success in these populations.

The mean number of visits per flower per day in each population (0.035, 0.274, and 0.775 for LH, WR, and IC) is positively associated with the frequency of late SC in these populations and with outcrossing rates (mean $t = 0.06$, 0.37, and 0.69, respectively; Goodwillie, 2000; Goodwillie and Ness, 2005). A possible interpretation is that the outcrossing rate has evolved in response to pollinator availability. When pollinators are scarce, there is a strong advantage to selfing via reproductive assurance (Baker, 1955). Under this scenario, we would also expect early SC and pollination-triggered senescence to be advantageous because they would reduce the maintenance cost associated with open flowers. Low visitation also may be a consequence, rather than a cause, of higher selfing rates if the evolution of selfing is accompanied by reduction of displays or rewards for pollinators. In the three field sites used in this study, corolla tube length, corolla lobe length, and pollen production are positively correlated with the outcrossing rate and the frequency of late SC (Goodwillie and Ness, 2005; C. Goodwillie, unpublished data). Regardless of its historical role in the evolution of selfing, the current pollinator environment strongly influences the potential magnitude of pollen discounting incurred by selfing-induced flower senescence.

Despite considerable theoretical interest in the evolution of flower longevity, relatively little work has focused on relationships between flower longevity and mating systems (Karle and Boyle, 1999; Sato, 2002). Primack (1985) observed that selfing species generally have shorter-lived flowers than

outcrossing species of the same genus or family. He theorized that selfing species may benefit from pollinating quickly and moving on to fruit development, whereas an outcrossing species gains fitness by allowing flowers to remain open longer to increase the probability of visitation. Sato (2002) showed that the timing of autonomous self-fertilization in *Impatiens hypophylla* mediates both flower longevity and the rate of selfing in populations. To our knowledge, however, selfing-induced flower senescence has not been considered previously as a selective force in mating system evolution.

Our study involved a species with an unusual form of variation in the timing of self-compatibility. However fertilization-triggered senescence is a common phenomenon that has been observed in many species (Gori, 1983; O'Neill, 1997; Yasaka et al., 1998; van Doorn, 2002; Rathcke, 2003; Underwood et al., 2005; Rogers, 2006), and any such species with early autonomous selfing might experience pollen discounting as a result of selfing-induced flower senescence. Moreover, individuals with a mutation promoting autonomous selfing in a largely outcrossing population may encounter intermediate pollinator visitation rates leading to pollen discounting. As such, we suggest that the selective importance of this phenomenon may be widespread. The potential role of selfing-induced senescence in limiting pollen export is an example of how pollination mechanisms and details of floral biology can affect mating outcomes, an idea emphasized in plant mating system theory (Lloyd and Schoen, 1992; Harder and Wilson, 1998).

In our field study, logistical constraints prevented us from determining the timing of SC of individual plants. Although populations differ in the frequency of early and late SC, our samples are likely to have included individuals of both types and therefore do not provide a direct comparison of phenotypes. Future experiments may be improved by establishing experimental gardens of known phenotypes in the field to examine rates of senescence in natural pollinator environments. A complementary approach to assessing this source of pollen discounting would involve genetic marker studies that compare outcross paternity of early and late selfing phenotypes in experimental arrays (Kohn and Barrett, 1994; Chang and Rausher, 1998; Fishman, 2000).

We have shown that the timing of SC, which is strongly associated with the rate of selfing, affects the longevity of flowers in *L. jepsonii*. Pollen presentation throughout anthesis and pollinator visitation rates for some populations of *L. jepsonii* suggest that flower longevity can limit opportunities for pollen export. Taken together, these lines of evidence suggest a potential cost of autonomous selfing that warrants further consideration in this and other studies of mating system evolution.

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