

EVOLUTION OF THE MATING SYSTEM IN A PARTIALLY SELF-INCOMPATIBLE SPECIES: REPRODUCTIVE ASSURANCE AND POLLEN LIMITATION IN POPULATIONS THAT DIFFER IN THE TIMING OF SELF-COMPATIBILITY

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Self-fertilization can provide reproductive assurance during periods of low or unreliable pollinator visitation. Therefore, periods of low pollen receipt may favor evolutionary shifts from cross-fertilization to self-fertilization. Although reproductive assurance is hypothesized to be important in mating system evolution, it has been quantified in relatively few species. *Leptosiphon jepsonii* shows variation in the mode of selfing, with transient self-incompatibility conferring delayed selfing seen in some individuals and early self-compatibility present in others. Autofertility, reproductive assurance, and pollen limitation were quantified in three populations of *L. jepsonii* that vary in the timing of self-compatibility and rate of self-fertilization. Plants in all three populations were capable of high seed set through autonomous self-fertilization, and selfing provided significant reproductive assurance in two populations, including one with the highest frequency of delayed selfing. Confidence intervals revealed no difference in reproductive assurance among populations. We conclude that both early and delayed self-compatibility can confer reproductive assurance and alleviate pollen limitation in this species. Results from this study are integrated with previous studies on the same three populations. We synthesize data on inbreeding depression, outcrossing rates, and floral biology for a comprehensive evaluation of the factors affecting the evolution of mixed mating in *L. jepsonii*.

Keywords: delayed selfing, mixed mating, pollen limitation, reproductive assurance, self-compatibility.

Introduction

The evolution of self-fertilization from cross-fertilization is proposed to be one of the most common evolutionary shifts in angiosperms (Stebbins 1974). The advantages of self-fertilization include both genetic and ecological factors. Two opposing genetic forces, the automatic transmission advantage of selfing and inbreeding depression, have been considered extensively (Fisher 1941; Nagylaki 1976; Lande and Schemske 1985; Charlesworth and Charlesworth 1987). In simple models of mating system evolution, inbreeding depression, the reduced fitness of inbred offspring, is expected to select for outcrossing if it exceeds 50%, which is the advantage conferred by transmission of genes through self-fertilization (Lloyd 1979). However, ecological factors can also be important in the evolution of plant mating systems (Lloyd and Schoen 1992; Barrett and Harder 1996). For example, the deposition of pollen by biotic and abiotic vectors is affected by a variety of ecological factors and can influence the evolutionary trajectory of plant mating systems.

Pollen limitation refers to a seed set that is limited by the amount and quality of pollen received (Knight et al. 2005). Consequently, pollen limitation is cited as one of the major reasons why angiosperms often produce fewer seeds than the total number of ovules (Bierzychudek 1981; Sutherland 1986; Burd 1994). Given that pollinator visitation is often variable, it is not surprising that a large percentage of flowering plants

(~62%) have been shown to be pollen limited (Burd 1994; Knight et al. 2005). Experimental evidence for pollen limitation is provided when pollen supplementation produces larger seed sets than does open pollination.

Pollinator visitation has been shown to vary in space and time (Eckhart 1992; Kalisz and Vogler 2003; Price et al. 2005; Kennedy and Elle 2008). In the context of low or unreliable visitation, self-fertilization that occurs without the aid of pollinators (autonomous selfing) can provide reproductive assurance of seed set (Darwin 1876; Baker 1955; Lloyd 1992) and alleviate pollen limitation (Larson and Barrett 2000). Therefore, periods of low pollen receipt can favor evolutionary shifts to self-fertilization from cross-fertilization. In fact, selfing populations are commonly found at the edge of species ranges or in extreme habitats, where they may receive fewer pollinator visits (Stebbins 1957; Jain 1976; Schoen 1982; Wyatt 1986). This trend is evidence of the role of reproductive assurance in the evolution of self-fertilization, as is the finding from comparative studies of lower pollen limitation in selfing than in outcrossing populations or species (Piper and Charlesworth 1986; Larson and Barrett 2000; Goodwillie 2001). Reproductive assurance can be quantified directly by a comparison of seed sets in unmanipulated and emasculated flowers, both of which are left open to pollinators. The difference in seed set represents the contribution of self-fertilization to female fitness (Cruden and Lyon 1989; Schoen and Lloyd 1992). Although reproductive assurance has been hypothesized to contribute to the evolution of selfing in many species, it has been quantified by this method in a limited number of studies to date (Eckert and Schaefer 1998; Kalisz et al. 2004;

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van Kleunen et al. 2007; Jacquemyn and Brys 2008; Kennedy and Elle 2008; Zhang and Li 2008; reviewed in Eckert et al. 2006).

Given these evolutionary consequences of selfing, theory predicts that delayed selfing—selfing that occurs after opportunities for outcrossing have occurred—is advantageous because it provides reproductive assurance when pollinator visitation is low but does not diminish male or female success through cross-pollination (i.e., it avoids costs of pollen and seed discounting; Lloyd 1979, 1992). Selfing that occurs before or concurrent with outcrossing opportunities (termed prior and competing selfing, respectively) can also provide reproductive assurance but, in contrast to delayed selfing, is likely to incur costs of inbreeding depression when outcrossing is possible (Lloyd 1979, 1992). Compared with delayed selfing, however, prior selfing might provide benefits if stigma receptivity or gamete viability declines with flower age, as has been shown for some species (Smith-Huerta and Vasek 1984; Morse 1987; Thomson and Thomson 1992; Castro et al. 2008), or if there are costs associated with flower maintenance prior to fertilization (Ashman and Schoen 1994, 1997). Although the conditions under which delayed versus prior selfing evolve from outcrossing have been considered (Lloyd 1979, 1992), evolutionary transitions between these modes of selfing have received little theoretical or empirical attention (Armbruster et al. 2002).

Transitions between selfing modes might have important implications for the evolution and maintenance of mixed-mating systems, in which fertilization occurs by both self and cross-fertilization. Some theory indicates that, because inbreeding depression can be purged with self-fertilization, mixed mating is expected to be evolutionarily unstable (Lande and Schemske 1985). However, intermediate outcrossing rates have been found for a large number of angiosperms, and subsequent models that incorporate additional genetic or ecological parameters have identified conditions under which mixed mating can be maintained (reviewed in Goodwillie et al. 2005). Mechanisms of delayed selfing promote mixed mating with variable pollination because they allow for opportunities for outcrossing before selfing occurs (Cruden and Lyon 1989). In contrast, floral traits that promote selfing before or during outcrossing are expected to yield higher selfing rates. Therefore, the evolutionary transition from delayed to prior selfing is likely to be associated with an increase in the selfing rate and, if so, the conditions that allow this transition are conditions under which mixed mating is unstable.

In *Leptosiphon jepsonii*, a California annual plant species, mixed mating and delayed selfing is conferred by a modified, transient form of self-incompatibility (SI). In this breeding system, which is similar to mechanisms of partial SI described in species of *Campanula* (Stephenson et al. 2000) and *Solanum* (Mena-Ali et al. 2008), flowers initially reject self pollen but become self-compatible (SC) 1–2 d later. This form of partial SI is the most common phenotype in *L. jepsonii*, but, in nearly all populations sampled to date, some individuals are SC when flowers first open (Goodwillie et al. 2004; C. Goodwillie, unpublished data), which is expected to promote prior or competing selfing. The presence of these early-selfing variants in populations of *L. jepsonii* warrants investigation, given the proposed advantages of delayed selfing. A possible selective

explanation for the evolution of earlier selfing is that transient SI does not allow complete reproductive assurance through selfing (Goodwillie and Ness 2005). If so, transient SI could promote more pollen limitation than could early selfing, and a further breakdown of the SI system might be favored under pollinator-limited conditions.

To gain insight into the evolutionary factors that have shaped this variation, we have studied three populations that span a range in the frequency of early-SC individuals. We have compared these populations in previous investigations of inbreeding depression, outcrossing rates, and other parameters that are relevant to mating system evolution (Goodwillie and Ness 2005; Goodwillie and Knight 2006; Weber and Goodwillie 2007). Here we present the final phase of our comparative study of these populations, which comprises the results of floral manipulations that address the following questions in populations that differ in the extent of early versus delayed SC: (1) What is the rate of autofertility, that is, the potential for autonomous selfing to achieve seed set? Autofertility could be affected either by factors that limit self-pollen deposition or by pollen-pistil incompatibility interactions. (2) Is autonomous selfing providing reproductive assurance? The extent to which selfing provides reproductive assurance will depend on both the capacity for autonomous selfing and the rate of pollinator visitation. (3) Is seed set pollen limited? Populations that exhibit significant reproductive assurance are expected to show little pollen limitation of seed set.

Furthermore, we integrate these data with the results of our previous studies of the same three contrasting populations. Information on mating systems, inbreeding depression, floral biology, pollinator visitation, and the results of floral manipulations presented here are synthesized to draw inferences about the factors affecting the trajectory of mating system evolution in *L. jepsonii*.

Material and Methods

Study Species and Populations

Leptosiphon jepsonii (Schemske and Goodwillie) Porter and Johnson is a small annual plant restricted to Lake, Napa, and Sonoma counties, in the California North Coast Range. The corolla is salverform, and its tube is 20–36 mm long. Flowering occurs from early April through May, and fruits mature after 3 wk. The species is visited and pollinated primarily by bee flies (Bombyliidae; order Diptera) and, to a lesser extent, small bees (order Hymenoptera). In most plants, no more than one flower is presented at a time; therefore, between-flower self-pollination (geitonogamy) does not generally occur. *Leptosiphon jepsonii* shows genetically based variation within and among populations in the timing of SC (Goodwillie and Ness 2005; C. Goodwillie, unpublished data). Most plants exhibit delayed self-fertilization via transient SI, in which stigmas become SC on the second or third day of anthesis (Goodwillie et al. 2004). Transient SI appears to be a modified form of the SI system that is inferred to be ancestral in the genus (Goodwillie 1999; Goodwillie et al. 2004). Populations of *L. jepsonii* also contain individuals that are SC immediately upon flower opening and remain so

throughout anthesis. Hereafter, we refer to these variants as delayed-SC (transient SI) and early-SC phenotypes.

Populations used in this study occurred at the Wantrup Reserve (WR; Napa County), near Lake Hennessey (LH; Napa County), or along Ida Clayton Road (IC; Sonoma County). The flowering phenology of the three sites was somewhat staggered but overlapping, with the WR population flowering first and the IC population flowering last. The different populations have similar habitats, occurring on open grassy slopes, although they span a range of elevations (LH: 98 m; WR: 274 m; IC: 390 m). Population size estimates ranged from 15,000 (WR) to 38,000 (IC) plants. Importantly for this study, the populations vary in the frequency of SC phenotypes and in a number of other important floral and mating system parameters (table 1).

Pollination Protocol

All pollination experiments were performed in spring 2006. A total of six floral treatments were used in each study population. Plants that were open to pollinators were placed into two treatment groups: emasculated (1) and unmanipulated (2). Additionally, plants over which we installed a pollinator exclusion tent were placed into four treatment groups: emasculated (3), unmanipulated (4), hand-outcrossed (5), and hand-selfed (6). Pollinator exclusion tents were made of tulle (pore size, 1 mm × 1 mm). All emasculations were performed

in the bud on the day before anthesis, before anthers had dehisced. Hand-outcrossed flowers were first emasculated to prevent self-pollen deposition; upon flower opening, stigmas were brushed with anthers from two other plants. Hand-selfed flowers were brushed with self pollen upon flower opening. Because *L. jepsonii* individuals produce relatively few flowers, with only zero to two flowers open in general on any given day, it was not possible to replicate all treatments on an individual; a single floral treatment was performed on each individual, and each assignment was chosen haphazardly within the experimental area. Each treatment was replicated on ~30 individuals at WR and IC. At LH, the sample size was increased to ~40 individuals because we anticipated that variation in weather conditions early in the season might add noise to the data set. The calyces of each experimental flower were marked with colored ink, and, at maturity, fruits were collected and seeds were counted. ANOVA was performed with Games-Howell post hoc tests (equal variances not assumed) to examine significant differences in seed number per flower among treatments within each population (SPSS 2008).

Comparisons among treatment means were used to gather a range of information for each population. The emasculated tent treatment served as a control to test the effectiveness of the pollinator exclusion tent and of the emasculation technique. The comparison between hand-selfed and tented unmanipulated treatments tested whether deposition of self pollen limits self seed set. Comparison of hand-outcrossed seed set

Table 1

Mating System, Pollination Biology, and Floral Parameters for Three Populations of *Leptosiphon jepsonii*

Parameter	LH	WR	IC	Sample size per population
Frequency of SC phenotypes ^a				30 plants, pollen tubes assayed on three flowers per plant
SC on day 1 (early SC)	.71	.16	.10	
SC delayed until day 2	.23	.78	.30	
SC delayed until day 3	.03	.03	.35	
SC delayed until day 4, or always SI	.03	.03	.25	
Mean number of days SI	.38	.93	1.75	
Mean outcrossing rate ^{a,b}	.06 (.08)	.37 (.15)	.69 (.01)	40–52 maternal families, mean progeny per family = 14.4
Cumulative inbreeding depression ^{c,d}	.297	.495	.501	10 maternal families, 12 progeny per family of each cross type
Pollinator visitation rate (number of visits per flower per day) ^e	.035 (.06)	.28 (.34)	.78 (.82)	8 h of pollinator observations
Corolla tube length (mm) ^a	27.7 (3.23)	32.7 (3.26)	39.9 (4.35)	30 plants, trait measured on three flowers per plant
Corolla lobe length (mm) ^a	3.8 (.35)	4.3 (.33)	4.2 (.38)	30 plants, trait measured on three flowers per plant
Stigma-anther separation (mm) ^{a,e}	-.28 (.30)	-.02 (.31)	+0.09 (.12)	30 plants, trait measured on three flowers per plant
Mean floral longevity (days) ^f	1.55 (.55)	2.20 (.76)	2.51 (.94)	45 plants, trait measured on one flower per plant

Note. Standard deviations shown in parentheses. IC = Ida Clayton Road, LH = Lake Hennessey, SC = self compatible, SI = self-incompatibility, WR = Wantrup Reserve.

^a Goodwillie and Ness 2005.

^b Standard deviation of estimates for 2 yr.

^c Goodwillie and Knight 2006.

^d Cumulative value is derived by multiplying population means for various life stages, so standard deviation cannot be calculated.

^e Negative number indicates vertical overlap; positive number indicates separation.

^f Weber and Goodwillie 2007.

with tented unmanipulated seed set provided a measure of autofertility, or the proportion of maximum (hand-outcrossed) seed set that can be produced by autonomous self-fertilization. Tented unmanipulated seed set can be achieved through autonomous selfing, unless inability for self-pollen deposition and/or SI prevents fertilization. Reproductive assurance was assessed by comparing open-pollinated unmanipulated seed sets with open-pollinated emasculated seed sets, because additional open-pollinated unmanipulated seed set is attributable to the ability to self-pollinate. The outcrossed seed set compared with the open-pollinated unmanipulated seed set is a test of pollen limitation, because outcrossed seed set is not limited by the amount or quality of pollen received.

Reproductive parameters were calculated for each population from treatment means as follows: autofertility (AF; seed set of tented unmanipulated flowers/seed set of hand-outcrossed flowers), reproductive assurance (RA; $1 - [\text{seed set of open-pollinated emasculated flowers/seed set of open-pollinated unmanipulated flowers}]$), and pollen limitation (PL; $[\text{seed set of hand-outcrossed flowers/seed set of open-pollinated unmanipulated flowers}] - 1$). To test for differences among populations in these mating system parameters, 95% confidence intervals were determined. These were achieved using a resampling technique in which observations were randomly sampled with replacement 10,000 times, employing the "Resampling Stats for Excel 3.0" add-in (Resampling Stats, Arlington, VA).

Results

Floral treatments had a significant effect on seed set per flower in all populations (tables 2, 3; fig. 1). In each population, the emasculated tent treatment produced a mean seed set that was <1.0 , indicating that both the pollinator exclusion tent and the emasculations were largely effective. In none of the populations did the hand-selfed treatment produce significantly greater seed set than the tented unmanipulated treatment, which suggests that stigma-anther separation does not reduce the ability of flowers to self-fertilize autonomously. Autofertility was high in all populations, as seed sets produced by outcrossed treatments and by tented unmanipulated treatments did not differ significantly. In tests for reproductive assurance, the ability to self-pollinate significantly increased seed set at LH and IC but not at WR. Results for pollen limitation also differed among populations; the two populations that exhibited significant reproductive assurance showed no evidence of pollen limitation (table 2; fig. 1). Open-pollinated unmanipulated flowers produced significantly lower seed sets than did hand-outcrossed flowers at WR, and reproductive as-

surance was not significant; however, no significant pollen limitation was observed at LH or IC, where significant reproductive assurance had been observed.

Confidence intervals for autofertility, reproductive assurance, and pollen limitation that were based on bootstrapped resampling were relatively large in most cases, reflecting considerable variability in the data (table 3). Estimates of autofertility and reproductive assurance were largely overlapping in the three populations. Only for pollen limitation were populations found to differ on the basis of the results of resampling; the confidence interval for the WR population did not overlap with that of the much lower estimate for the IC population.

Discussion

Reproductive Assurance, Pollen Limitation, and Autofertility in Leptosiphon jepsonii

This study presents evidence that self-fertilization provides reproductive assurance in *Leptosiphon jepsonii*. In two of the three populations, seed set in emasculated open-pollinated flowers was significantly lower than it was in unmanipulated open-pollinated flowers, indicating a contribution by autonomous selfing. Within-flower selfing facilitated by pollinators could also contribute to increased seed set in unmanipulated flowers. Estimates of reproductive assurance in all three populations (table 3) were well above the median value of 0.2 found in a survey of similar emasculation studies in 29 angiosperm species (data from Eckert et al. 2006). Of particular note, reproductive assurance was high at IC, where the population is dominated by transiently SI individuals. It is often argued that partial SI evolved because it assures seed set when pollinator visitation is unreliable (Levin 1996; Stephenson et al. 2000). To our knowledge, however, this is the first study to use an experimental approach to quantify reproductive assurance by selfing in a partially self-incompatible species. Our results are also congruent with the finding of Fenster and Marten-Rodriguez (2007) that delayed-selfing mechanisms for reproductive assurance are often associated with specialized pollination; *L. jepsonii* flowers are visited predominantly by long-tongued flies.

Consistent with the finding that selfing provides reproductive assurance in *L. jepsonii*, autofertility was high in all populations. Inbreeding depression that acts early in development can lead to abortion of embryos and limit the contribution to seed set from self-fertilization. Thus, high autofertility in all of the populations studied implies both the ability to self-fertilize and the absence of early-acting inbreeding depression. These results are in agreement with previous work performed on the study populations that found little inbreeding depression at early life stages (Goodwillie and Knight 2006). On the basis of the comparison of hand-selfed and autonomously selfed flowers, stigma-anther separation, a trait that varies among the populations (table 1) and that has been shown to affect the mating system in other species (e.g., Schoen 1982; Robertson and Lloyd 1991; Holtsford and Ellstrand 1992; Brunet and Eckert 1998; Stone and Motten 2002), appeared to have no effect in these populations on the ability to self autonomously.

Table 2

ANOVA Results for the Effect of Floral Treatment on Mean Seed Set per Flower in Three Populations

Source of variation	df (error)	Mean square (error)	F
Lake Hennessey	5 (219)	266.03 (11.28)	23.58*
Wantrup Reserve	5 (164)	205.64 (11.46)	17.94*
Ida Clayton Road	5 (170)	91.61 (9.90)	9.25*

* $P < 0.001$.

Table 3
Values (95% Confidence Intervals) Based on Bootstrap Resampling for Autofertility, Reproductive Assurance, and Pollen Limitation in Each Experimental Population

Parameter	Population		
	Lake Hennessey	Wantrup Reserve	Ida Clayton Road
Autofertility	1.056 (.84–1.33)	1.055 (.75–1.50)	1.367 (.93–2.00)
Reproductive assurance	.557 (.37–.78)	.710 (.26–.90)	.485 (.24–.68)
Pollen limitation	.119 (–.30 to .56)	.563 (.33–4.23)	–.447 (–.51 to –.04)

Self-fertilization that occurs autonomously is predicted to alleviate pollen limitation because it decreases dependence on vectors for pollination, and selfing species were found to experience significantly reduced pollen limitation in a broad comparative study (Larson and Barrett 2000). Therefore, it is not surprising that our experimental results revealed a similar trend: the two populations that had significant reproductive assurance showed no significant pollen limitation. The results of this study are generally consistent with our previous findings, that seed set was not limited by pollen in two of three populations of *L. jepsonii* (Goodwillie 2001). In contrast, the earlier study found substantial and significant pollen limitation in populations of a closely related sympatric species with fully effective SI, *Leptosiphon parviflorus*. In one population of *L. parviflorus*, the seed set from open pollination was less than half of that in the pollen-supplemented treatment. Taken together, evidence for reproductive assurance and lack of pollen limitation in *L. jepsonii* and for substantial pollen limitation in an SI congener suggest that selection for reproductive assurance through selfing may have played a role in the evolution from complete to transient SI.

The finding of significant pollen limitation and no significant difference in seed set between emasculated and open-pollinated flowers at the WR population implies, at face value, that autonomous selfing was not effective in that population. However, the WR population showed the highest estimate of reproductive assurance based on the ratio of treatment means.

In addition, these findings are incongruent with the high autofertility estimated for the WR population. Inspection of the treatment means (fig. 1) reveals that open-pollinated unmanipulated flowers produced significantly fewer seeds than did unmanipulated flowers in the tent. This result is difficult to interpret biologically and suggests some source of experimental error, such as the pollinator tent positively influencing seed set, that could affect the estimates of both pollen limitation and reproductive assurance in the WR population.

An additional and important caveat of our study concerns an untested assumption that emasculation does not affect attractiveness to pollinators. The generally low rate of pollinator visitation, especially in the LH population (table 1), made it unfeasible to carry out a rigorous test for this effect. If emasculated flowers receive fewer visits than unmanipulated flowers, estimates of reproductive assurance might be inflated; however, few studies using the emasculation method for measuring reproductive assurance have included this control (Eckert et al. 2006). On the basis of previous experimentation, we also made the assumption that experimental techniques of hand pollination and emasculation do not cause overall damage to individuals of *L. jepsonii*. However, in the IC population, flowers seemed to be particularly sensitive to being handled (J. Weber, personal observation). This might account for the finding that the hand-outcrossed treatment, which was expected to yield the maximum seed set, yielded a lower mean seed set than did open pollination.

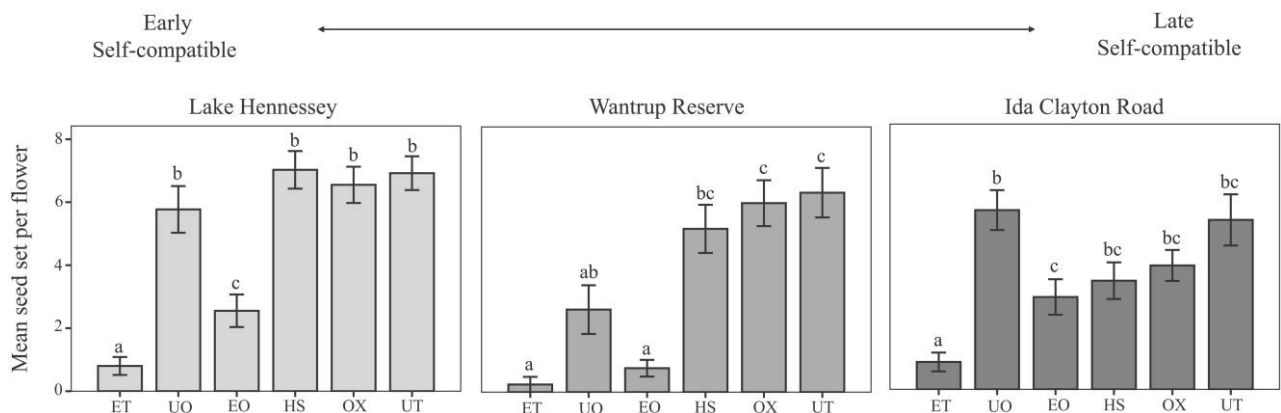


Fig. 1 Mean seed set per flower at Lake Hennessey, Wantrup Reserve, and Ida Clayton Road from six floral manipulations. ET = emasculated tent, UO = unmanipulated open, EO = emasculated open, HS = hand selfed, OX = hand outcrossed, and UT = unmanipulated tent. Letters above bars indicate treatments that were significantly different in post hoc tests. Error bars represent ± 1.00 SE. At Lake Hennessey, there were $N = 40$ individuals per treatment; at Wantrup Reserve and Ida Clayton Road, there were $N = 30$ individuals per treatment.

Artificially low values for the outcrossed seed set caused by handling damage would have the effect of inflating values for autofertility and decreasing estimates of pollen limitation. Finally, since manipulations to flowers have the potential to draw resources from other flowers or fruits, treatment effects at the single-flower level may be somewhat inflated relative to what would be observed at the whole-plant level (Zimmerman and Pyke 1988; Knight et al. 2006).

*Evolution of the Mode of Selfing in L. jepsonii:
Integration of Mating System Parameters*

The combined results of a series of studies allow us to begin to assess the adaptive value of modes of selfing in *L. jepsonii* and to consider the stability of its mixed-mating system. The timing of SC varies widely in *L. jepsonii*, with some populations, such as IC, that are dominated by delayed SC and with others containing a high frequency of early-SC phenotypes. What can our knowledge of other mating system parameters tell us about the evolutionary trajectories of these populations and the factors that drive transitions between different modes of selfing?

We expect delayed selfing to be favored over prior or competing selfing because it allows outcrossing when pollinators are present, avoiding the costs of pollen or seed discounting (Lloyd 1979, 1992). Our data do suggest that delayed SC compared with early SC promotes outcrossing: as estimated from isozyme analysis of progeny arrays, we found a positive trend (although not a statistically significant one) among the populations between the frequency of delayed-SC phenotypes and the outcrossing rate (table 1; Goodwillie and Ness 2005). We note, however, that because variation among populations in the visitation rate is confounded with variation in the mode of selfing (table 1), we cannot tease apart the effects of these two variables on the outcrossing rate without studying the consequences of individual variation in the timing of SC in a common pollinator environment.

The strength of the hypothesized benefit of delayed selfing over early selfing, however, will depend on the rate of pollinator visitation and the magnitude of the cost of selfing through inbreeding depression. In the LH population, visitation was negligible and inbreeding depression was found to be low (table 1). Together, these data suggest that a delay in self-fertilization confers little advantage in this population related to its effect on the rate of outcrossing. Conversely, in both the WR and the IC populations, reasonably high visitation rates provide opportunities for outcrossing, and inbreeding depression estimates indicate a cost of selfing that balances the 50% automatic transmission advantage. Therefore, selection to maximize outcrossing might act against early-SC variants in the WR and IC populations and serve to maintain mixed mating and delayed selfing. In contrast, as discussed by Eckert et al. (2006), similar cost-benefit approaches used in two other well-studied species, *Aquilegia canadensis* and *Collinsia verna*, cannot explain the maintenance of their mixed-mating systems: although reproductive assurance by selfing alleviates pollen limitation in both species, inbreeding depression estimates appear to be too high in *A. canadensis* (0.93; Herlihy and Eckert 2002) to maintain selfing in the population and too low in *C. verna* (~ 0.10 ; Kalisz et al.

2004) to maintain a substantial degree of outcrossing. Ironically, reproductive-assurance and inbreeding-depression levels in *L. jepsonii* appear to be more likely to promote stable mixed mating in the IC and WR populations, since the inbreeding depression cost of selfing is comparable with its genetic transmission advantage. Yet the populations contain substantial frequencies of early-SC variants, which suggests that they are not fully resistant to invasion by alleles that promote higher selfing.

Compared with prior selfing, delayed selfing is also expected to promote greater outcross siring success because pollen is used for selfing only after opportunities for pollen dispersal are taken, avoiding the cost of pollen discounting (Lloyd 1992). We have not yet directly quantified the effects of delayed versus early SC on male outcrossing fitness. In a previous study, however, we proposed an additional source of pollen discounting related to flower longevity: because fertilization induces flower senescence in *L. jepsonii*, as has been shown to occur in many plant species (Stead 1992), early SC might shorten flower longevity and therefore reduce opportunities for pollen dispersal and male outcrossing success (Weber and Goodwillie 2007). Consistent with this hypothesis, mean longevity of unmanipulated flowers was lowest in the predominantly early-selfing LH population and highest in the IC population dominated by delayed SC (table 1), although flower longevity varied little across populations in both hand-outcrossed and emasculated treatments (Weber and Goodwillie 2007). Delayed selfing could be selected to minimize pollen discounting by either of these means, but the magnitude of the advantage will depend on pollinator visitation. With low visitation and few opportunities for outcrossing, effects of prior selfing on the amount of pollen available for dispersal and on flower longevity will have little consequence for male success. Therefore, this selective effect is likely to be important only for the IC and WR populations, in which we observed reasonably high visitation.

The arguments above suggest that the advantages of delayed selfing over prior selfing that are associated with reducing pollen discounting and avoiding inbreeding depression are expected to be minimal with low pollinator visitation and low inbreeding depression. However, explaining the presence of early-selfing phenotypes at high frequencies in LH and also at moderate frequencies in WR and IC appears to require a selective benefit to early SC. The current study provides no support for the hypothesis that early SC is selected because it is more effective than delayed SC in providing reproductive assurance; autofertility was uniformly high in populations that otherwise differed dramatically in the frequency of early- versus delayed-SC phenotypes. Reproductive assurance was evident even in the population with the highest frequency of delayed SC, and confidence intervals for this parameter in the three populations were widely overlapping. On the basis of these data, then, differences in reproductive assurance do not appear to explain the maintenance of early-SC variants. These results contrast, however, with a previous greenhouse study of plants from 17 populations that showed a trend toward higher autofertility in plants with early SC (Goodwillie and Ness 2005). The reasons for the discrepancy are not clear but may be related to differences in field and greenhouse growth conditions. Larger field studies addressing

the consequences of individual variation in mode of selfing are warranted.

Alternatively, a loss of reproductive success with floral age, which has been shown to occur in other species through changes in pollen viability (Smith-Huerta and Vasek 1984; Thomson and Thomson 1992; Rodriguez-Riano and Dafni 2007) and pistil receptivity or viability (Morse 1987; Castro et al. 2008) or through costs of maintaining flowers (Ashman and Schoen 1997), might select for early selfing. However, in a study of plants in the WR population (J. Weber, unpublished data), mean seed set was not found to differ in flowers that were cross-fertilized on the first, second, and third days of anthesis in both field ($N = 30$, $P = 0.335$) and greenhouse ($N = 40$, $P = 0.687$) experiments, indicating that the timing of ovule fertilization does not appear to affect reproductive success. Additionally, pollen viability does not decrease from the first to the second day of anthesis (Weber and Goodwillie 2007). Thus, the selective factors that account for the presence of early-SC variants in many populations remain unknown.

A final hypothesis for their presence demands future consideration: higher selfing (through early SC) in the LH population might be selected because it limits production of low-fitness hybrids, as has been proposed in a number of other species (Levin 1972; Fishman and Wyatt 1999; Eckert et al. 2006). At the LH site, *L. jepsonii* occurs in direct contact with *Leptosiphon androsaceus*, a close relative that is self-incompatible and large flowered. Hybrids have been identified at the site, and experimentally produced F_1 hybrids have low fertility (J. Ness, unpublished data). Intriguingly, the three other populations of *L. jepsonii* that co-occur with either *L. androsaceus* or *Leptosiphon acicularis* also have unusually high frequencies of early SC. This hypothesis merits further consideration; however, it cannot account for the substantial frequency of early SC found in many other populations of *L. jepsonii* (Goodwillie and Ness 2005).

Finally, the interacting evolutionary causes and consequences of mating system evolution must be considered in order to understand the divergent evolutionary trajectories of populations of *L. jepsonii*. Differences among the LH, WR, and IC populations in flower size dimensions (corolla lobe and tube length; table 1) suggest that the higher rates of selfing that accompany a shift to early SC have resulted in selection to reduce allocation to attractive structures, a trend that is observed in other taxa with variation in outcrossing rate (Wyatt 1986; Ritland and Ritland 1989; Lyons and Antonovics 1991; reviewed in Brunet 1992). Although differences in pollinator abundance at these sites might have caused the initial divergence in mating systems, the evolution of reduced

floral display in the LH population is likely to further limit the rate of visitation, with cascading effects of mating system variation as discussed above. We have not directly quantified the effect of reduced floral display in *L. jepsonii*; however, floral display and size have been found to impact pollinator visitation in a number of other species (Vaughton and Ramsey 1998; Thompson 2001; Elle and Carney 2003; Kennedy and Elle 2008). Differences in the selfing rate might also have consequences for the evolution of inbreeding depression, as suggested by a comparison of the three populations (table 1; discussed in Goodwillie and Knight 2006). With higher rates of selfing in the LH population, perhaps initially caused by lower pollinator visitation and then further promoted by the evolution of early-SC phenotypes, genetic load has been purged to some extent, diminishing the strength of selection for traits that promote outcrossing.

Conclusion

Self-fertilization appears to alleviate pollen limitation in many species, but experimental tests of reproductive assurance have been performed for relatively few species. We have demonstrated that selfing increases seed set in *L. jepsonii*, providing the first explicit experimental test for reproductive assurance in a partially SI species. In addition, our results suggest that the presence of early-SC phenotypes in all populations cannot be explained by a selective advantage of assured seed set when pollinator visitation is variable. Moreover, examination of potential costs of delaying fertilization shows no decline in the fitness of female and male gametes with flower age. As discussed above, further understanding of the factors affecting the evolution of the timing of self-fertilization in *L. jepsonii* will require studies of individual phenotypic variation. For example, the use of known phenotypes (early or delayed SC) in experimental arrays would allow for a direct comparison of the fitness consequences of variation in the mode of selfing.

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