

College of the Holy Cross

CrossWorks

Biology Department Faculty Scholarship

Biology Department

1-1-2004

Correlated Evolution of Dichogamy and Self-Incompatibility: A Phylogenetic Perspective

Matthew B. Routley
University of Guelph

Robert I. Bertin
College of the Holy Cross, rbertin@holycross.edu

Brian C. Husband
University of Guelph

Follow this and additional works at: https://crossworks.holycross.edu/bio_fac_scholarship

Repository Citation

Routley, Matthew B.; Bertin, Robert I.; and Husband, Brian C., "Correlated Evolution of Dichogamy and Self-Incompatibility: A Phylogenetic Perspective" (2004). *Biology Department Faculty Scholarship*. 8. https://crossworks.holycross.edu/bio_fac_scholarship/8

This Article is brought to you for free and open access by the Biology Department at CrossWorks. It has been accepted for inclusion in Biology Department Faculty Scholarship by an authorized administrator of CrossWorks.

CORRELATED EVOLUTION IN FLORAL MORPHOLOGY AND THE TIMING OF SELF-COMPATIBILITY IN *LEPTOSIPHON JEPSONII* (POLEMONIACEAE)

Carol Goodwillie¹ and Jennifer M. Ness

Department of Biology, East Carolina University, Greenville, North Carolina 27858, U.S.A.

The timing of self-compatibility was found to vary within and among populations of *Leptosiphon jepsonii*, a narrowly distributed California annual species. In each of 17 populations, some individuals exhibited transient self-incompatibility, a phenomenon that confers delayed selfing, while others were fully self-compatible upon flower opening, allowing for concurrent self- and cross-fertilization. The frequency of initially self-compatible individuals varied significantly among populations, ranging from 3.2% to 71.0%. A comparison of outcrossing rate estimates for three populations was consistent with the hypothesis that early self-compatibility promotes higher selfing rates. Population means for each of three floral morphological traits were significantly correlated with a population index of self-incompatibility that reflects the frequency of fully self-compatible and transiently self-incompatible individuals. A high frequency of self-compatibility was associated with shorter corolla tubes, smaller corolla lobes, and greater overlap of stigma and anthers. The demonstration of this relationship among populations within a small geographic range suggests fine-scale local adaptation of mating system traits in *L. jepsonii*.

Keywords: delayed selfing, floral evolution, *Leptosiphon jepsonii*, mating system, Polemoniaceae, self-compatibility, self-incompatibility, outcrossing rate.

Introduction

One of the most common adaptive transitions in higher plants is the evolution of self- versus cross-fertilization (Stebbins 1950, 1974). For several decades, studies of intraspecific variation in the rate of self-fertilization (selfing) have provided insights into the evolution of this important character. For instance, the occurrence of selfing populations at range limits (Schoen 1982; Holtsford and Ellstrand 1989; Ohara et al. 1996), ecologically marginal areas (Moore and Lewis 1964), and xeric habitats (Lloyd 1965; Arroyo 1975; Solbrig and Rollins 1977; Dole 1992) have been interpreted as evidence that pollinator scarcity is a driving force in the evolution of self-fertilization. The majority of these studies concern self-compatible species (Moore and Lewis 1964; Ennos 1981; Wyatt 1984; Lyons and Antonovics 1991; Dole 1992; Motten and Antonovics 1992; Belaoussoff and Shore 1995; Karron et al. 1995; Routley et al. 1999; Lu 2000; Motten and Stone 2000). In contrast, comparatively few studies have addressed variation among populations in the presence or strength of homomorphic self-incompatibility (Lloyd 1965; Solbrig and Rollins 1977; Rick et al. 1979; Ohara et al. 1996; Lipow et al. 1999) despite the prevalence of this trait in angiosperms and its important role in plant reproductive strategies (de Nettancourt 1977).

We know relatively little about the breakdown of self-incompatibility (SI) and its relationship to the evolution of floral morphological traits. The transition from outcrossing to

selfing must begin with a breakdown of the SI system. In the few families in which SI has been well studied, its weakening or loss has been attributed to a variety of mechanisms, including mutations in the *S* locus, unlinked modifier loci, or duplications of the *S* locus (Nasrallah et al. 1992; McClure et al. 2000; Stone 2002). The loss of SI may be only a first step in the transition to a highly selfing strategy, however. The evolution of selfing often includes additional changes in a suite of morphological traits that reduce allocation to pollinator attraction and male function and increase rates of self-pollen deposition (Ornduff 1969). In comparisons of species with contrasting mating systems, cross-fertilization (outcrossing) has been shown to be associated with larger corolla size, higher pollen to ovule ratios, and greater spatial separation of stigma and anthers (Cruden and Lyon 1985; Ritland and Ritland 1989).

Theoretical work has shown that the evolution of mating systems can be influenced by functional dimensions of self-fertilization (Lloyd 1979, 1992; Lloyd and Schoen 1992). Three modes of autonomous selfing (self-fertilization without the aid of a pollen vector) have been distinguished based on the relative timing of selfing and outcrossing (Lloyd 1979; Lloyd and Schoen 1992). While all of these can provide reproductive assurance when pollinators are scarce, they differ in their potential costs. Selfing that occurs before outcrossing (prior selfing) or concurrent with outcrossing (competing selfing) can reduce the pollen or ovules available for cross-fertilization. In contrast, delayed selfing, in which selfing occurs after opportunities for outcrossing, does not incur such costs. Thus, the conditions favoring the selection for self-fertilization may depend on the mechanism and timing of self-fertilization (Lloyd 1979; Lloyd and Schoen 1992).

¹ E-mail goodwilliec@mail.ecu.edu.

Functional aspects of self-fertilization are increasingly considered in studies of plant mating systems (Leclerc-Potvin and Ritland 1994; Kalisz et al. 1999; Culley 2002; Herlihy and Eckert 2004), yet little empirical or theoretical work has addressed the transitions between modes of self-fertilization (Harder and Wilson 1998; Armbruster et al. 2002). Such studies may provide valuable insights into the evolutionary processes shaping plant mating systems.

Leptosiphon jepsonii provides a unique opportunity to study the evolution of pollen-pistil and floral morphological characters and transitions between different modes of selfing. The genus *Leptosiphon* (formerly *Linanthus* section *Leptosiphon*) in the family Polemoniaceae includes both SI and self-compatible (SC) species. Independent losses of SI have occurred in at least three lineages in the genus, with parallel evolution of reduced flower size in selfing taxa (Goodwillie 1999). *Leptosiphon jepsonii* holds particular interest because in several respects it is intermediate between the obligate outcrossing and nearly complete selfing observed in congeneric species. The species exhibits a transient form of SI in which stigmas become SC 24–48 h after flowers open. This phenomenon, which has been well described in *Campanula rapunculoides* (Stephenson et al. 1992; Vogler and Stephenson 2001), confers delayed selfing and intermediate outcrossing in *L. jepsonii* (Goodwillie 2000; Goodwillie et al. 2004). However, preliminary observations of several populations indicate that some individuals are immediately SC upon flower opening and suggest that the frequency of fully SC phenotypes varies among populations. Moreover, populations exhibit variation in floral traits associated with the mating system such as corolla size and stigma-anther position, with values ranging between those seen in fully selfing and obligately outcrossing congeners. Interestingly, this differentiation among populations occurs at a very small spatial scale, with all known populations *L. jepsonii* separated by less than 65 km.

The presence of local variation in the mode of selfing in *L. jepsonii* raises questions concerning the evolution of this trait. Is delayed selfing a stable adaptation maintained by selection or a transient phase in the evolution toward a more highly selfing species? What genetic or ecological factors are driving the dynamics of this mixed mating system? Here we present a phase of this investigation in which we carry out a common garden experiment to measure phenotypic variation in the timing of SC and the potential for autonomous selfing within and among populations throughout the species range, examine the relationship between the population outcrossing rate and the frequency of SC and transiently SI individuals, measure variation in floral traits within and among populations, and test for associations between morphological and pollen-pistil traits. In addition, we discuss some preliminary inferences concerning evolutionary processes acting in this dynamic system.

Material and Methods

Species and Site

The spring annual species *Leptosiphon jepsonii* has a restricted distribution, with present populations known from

only Napa and Sonoma Counties in the north coast ranges of California. Herbarium specimens indicate historical presence of populations in southern Lake County as well (Schemske and Goodwillie 1996). The species is found in open grassy areas of oak woodlands at elevations ranging from 98 to 735 m. The study sites included 17 of 19 known populations of *L. jepsonii* in Napa and Sonoma Counties (fig. 1).

The small plants have salverform corollas, with long slender corolla tubes and five corolla lobes. Corollas are either pink or white, with most populations fixed or nearly so for one flower color. Emasculated and unfertilized flowers can last up to 6 d, but flowers senesce about 1 d after fertilization occurs. Flowers are visited by bees (Bombyliids), which hover above the flowers while collecting pollen and nectar. Flowering occurs between late March and early June, and fruits mature and dehisce in about 3 wk. Ovule numbers range from 12 to 16 (C. Goodwillie, unpublished data).

Assays for Self-Incompatibility

Thirty-two plants from each study population were raised from seeds of plants collected in the field in spring 2002 or 2003. Each seed was taken from a different maternal plant separated from other plants by at least 1 m in the field. Seeds were germinated on moist filter paper at 4°C and then planted in Conetainers (Stuewe and Sons, Corvallis, OR) in standard potting soil. Plants were subirrigated continuously and watered once weekly with a mild solution of 12-55-6 commercial fertilizer. Seedlings were raised in a growth chamber, and mature plants were moved for the pollination experiments to a growth room with both artificial and natural light. Because of space and time limitations, all pollination experiments could not be conducted simultaneously; populations were tested sequentially between 2002 and 2004.

To assay plants for the presence and duration of SI, we counted pollen tubes in a series of experimental pollinations, each replicated on three flowers per plant. All pollinations were conducted between 0900 and 1030 hours, and styles were removed 3 h after pollination. Stigmas and styles were inspected for pollen tubes using aniline blue staining and epifluorescent microscopy, as described in a previous paper (Goodwillie et al. 2004). In the SI system of *Leptosiphon*, rejection of self-pollen occurs at the stigma surface and prevents pollen germination; thus, we quantified SC as the number of pollen tubes penetrating through the stigma lobe. Because the accuracy of counting diminished at high pollen tube densities, we set a maximum at 60 pollen tubes.

In previous experiments (Goodwillie et al. 2004), we applied self-pollen on the first, second, and third day that a flower was open and found that self-pollen tube counts often increased but never decreased with floral age. After verifying that developmental changes from SC to SI did not occur for several populations in the current study, we optimized our experimental protocol to discontinue self-pollinations once stigmas became SC.

Initially, two pollination treatments were applied to the stigmas of flowers that were open for the first day (hereafter day 1 flowers): pollen from two haphazardly chosen plants from the same population (cross-pollen) and self-pollen. We applied self-pollen to flowers of increasing age (day 1, day 2,

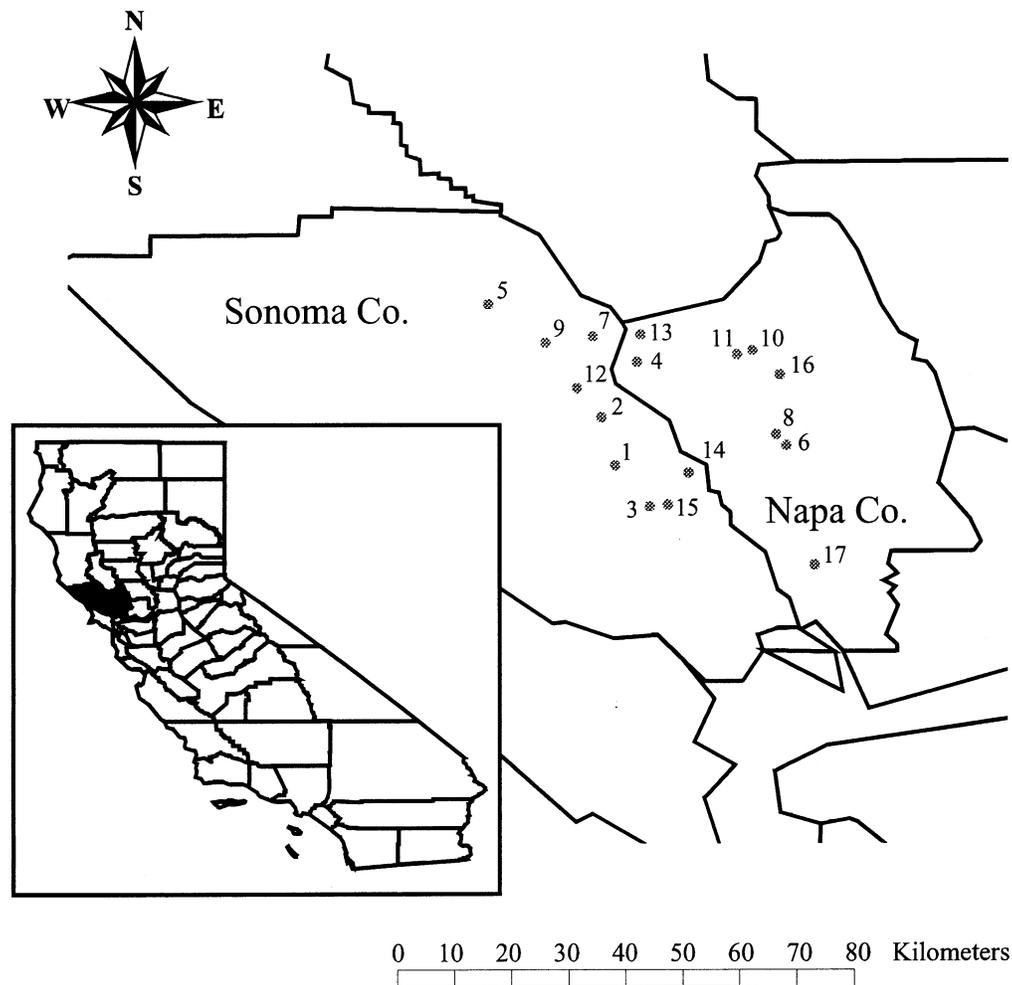


Fig. 1 Location of sampled populations in Napa and Sonoma Counties, California.

and day 3) until stigmas were found to be SC. We considered a mean pollen tube number >20 , which exceeds ovule number, to be SC. For self-pollinations on day 2 and day 3, flowers were emasculated on the morning that flowers first opened, and petals were marked with a colored dot on consecutive days to record flower age. In plants that did not produce self-pollen tubes by day 3, pollen was tested for viability by applying it to the stigmas of unrelated plants and staining for pollen tubes. In all of the treatments described above, fresh pollen from day 1 flowers was used. Previous experiments indicate that pollen age does not influence pollen-pistil interactions (Goodwillie et al. 2004).

Analysis. All analyses were carried out on SPSS v. 12 (SPSS 2003) except where noted. We used nested ANOVA to test for variation within and among populations in the timing of SC. As a measure of early SC versus transient SI, we used the number of self-pollen tubes in day 1 flowers. To improve the fit of the data to model assumptions, we expressed pollen tube number as a proportion of the maximum pollen tube count of 60 and arcsine transformed that value. Plants were nested within populations, and both were considered random

factors. Using methods described by Graham (2001), we calculated the magnitude of effect, or percent of total variance, found within plants, among plants within populations, and among populations. As another measure of variability among populations that takes into account more information on the timing of SC, we scored each plant as one of four SI classes on the basis of the time at which self-pollen tubes were first produced. Plants in class 1 produced >20 self-pollen tubes in day 1 flowers, plants in class 2 were SI on day 1 but produced >20 self-pollen tubes on day 2, and class 3 plants did not become SC until flowers were 3 d old. Plants with viable pollen that did not produce self-pollen tubes by day 3 were assigned to class 4. We carried out χ^2 analysis to test the null hypothesis that the frequency of SI class was independent of population source.

Seed Set

We examined the potential for seed set by facilitated and autonomous self-fertilization in growth room experiments. On a subset of 11 populations, we applied three pollination treatments on all plants used for the pollen tube study:

(1) outcross pollination as described above, (2) facilitated self-pollination, or (3) autonomous self-pollination (no manipulation). Pollinations for treatments 1 and 2 were carried out on day 1 flowers. Treatments were replicated on three flowers per plant, and calyces were marked with colored ink. We collected mature fruits and counted the number of seeds per flower.

Analysis. We used a mixed model ANOVA to test for differences in mean seed number per flower resulting from pollination treatments (fixed factor), variation among populations (random factor), and their interaction. For each experimental plant, a mean of three replicate flowers was used in the analysis. Scheffe post hoc tests were used to test for significant pairwise differences between treatments. On a combined data set of plants from the 11 populations, we tested for a correlation between the timing of SC and autonomous self seed set (treatment 3 above). We used self-pollen tube number in day 1 flowers as a measure of the timing of SC. To control for individual differences in ovule number or overall plant performance that could affect seed set, we used the difference between outcross and autonomous self seeds per flower for this correlation.

Floral Traits

On all experimental plants used for pollinations, we measured corolla tube length, corolla lobe length, and stigma-anther separation. Measurements were made with digital calipers and replicated on three flowers per plant. Stigma-anther separation was measured as the distance between the base of the stigma lobes and the top of the anthers. Thus, a negative number indicates vertical overlap of anthers and stigma lobes. ANOVA was used to test for variation in the three floral traits within and among populations. Population and plant were treated as random factors, and plants were nested within populations. We calculated the magnitude of effect, or percent of total variance, found within plants, among plants within populations, and among populations (Graham 2001).

Relationships between Self-Incompatibility and Floral Morphology

For each population, we calculated an overall SI index as the mean of SI class values (1–4) of individual plants. Thus, an SI index of 1 would indicate a population in which all individuals are SC on day 1. Note that this index differs from SI or SC indices used in other literature, which generally refer to a ratio of seed set in cross- and self-pollinated flowers (Becerra and Lloyd 1992). Using population means, we tested the correlation of SI index with each of the floral traits (corolla tube length, corolla lobe length, and stigma-anther separation). In separate analyses for each population, we tested for a correlation between individual plant means for floral traits and the mean number of self-pollen tubes in day 1 flowers. We also measured correlations among the three floral traits with population means and, in separate analyses for each population, with individual plant means. Significance levels were adjusted for multiple tests (Rice 1989).

It can be argued that populations in close proximity are more likely to exchange genes or share recent evolutionary

history than are distant populations. Thus, correlations among traits may reflect overall genetic similarity of neighboring populations, and as a result, adaptive arguments built on these trait associations may be spurious. To test for similarity in floral or SI traits corresponding to geographic distance, we employed Mantel tests using the software PASSAGE (Rosenberg 2001). We created a matrix of spherical distances among populations from latitudinal and longitudinal coordinates and tested for a correlation between the spatial distance matrix and distance matrices for SI index and overall floral morphology. Euclidean distance for floral morphology was calculated from population means for corolla tube length, corolla lobe length, and stigma-anther distance. For SI index, we calculated distance as the squared difference between population means. In addition, we carried out partial Mantel tests in which correlations between matrices are calculated while controlling for a third distance matrix (Smouse et al. 1986). This allows one to test whether apparent relationships between two factors can in fact be explained by the correlation of each to a third factor.

Mating System Analysis

We hypothesized that the timing of SC in populations would be related to the rate of outcrossing. That is, populations with a high frequency of individuals with transient SI were expected to have a higher outcrossing rate than those with predominantly early SC. We estimated mating system parameters in 2003 and 2004 for three populations (6, 7, and 16) that span most of the range in SI indices. Maternal seed families were collected haphazardly from these populations at the end of each season. Proteins were extracted from young seedlings using a sucrose buffer (0.1 M Tris base, 0.5 M sucrose, 0.02 M sodium bisulfite, pH 7.5; K. Ritland, personal communication). Using starch gel electrophoresis, we were able to resolve only two polymorphic loci: 6-phosphogluconate dehydrogenase (6PGD) and isocitrate dehydrogenase (IDH). Although all three populations contained at least two alleles at both loci, we found sufficient polymorphism for analysis in only one locus per population: 6PGD in populations 7 and 16 and IDH in population 6. Staining and running buffer (morpholine citrate) recipes followed Werth (1985). The number of maternal families per population and year ranged from 42 to 50, with a mean progeny number per family of 14.4.

Results

Assays for Self-Incompatibility

Final population sample sizes varied somewhat as a result of plant mortality (mean population sample = 30.7 plants, total = 519 plants). The pollen tube data confirmed the presence of transient SI in *Leptosiphon jepsonii*, as was reported for two populations in a previous study. In some individuals from each of 17 populations, self-pollen tube number in day 1 flowers was close to 0, while outcross pollen tubes grew readily upon flower opening. In 2- or 3-d-old flowers, however, the number of self-pollen tubes was considerably higher and often similar to that of outcross pollen tube number on

day 1 flowers. In all populations, some plants were largely SC immediately upon flower opening (>20 self-pollen tubes in day 1 flowers). Across all populations, a minority of plants produced intermediate numbers of pollen tubes in day 1 flowers. Of the total 519 plants assayed, 294 (57%) had a mean of five pollen tubes or fewer, 155 (30%) showed a mean greater than 20, and only 70 plants (13%) had means between five and 20. In a small number of plants, outcross pollen tube counts were low, which we interpreted as cross-incompatibility with pollen donors expressing the same SI genotype. Results for individual plants in population 14 (fig. 2) provide a representative sample of individual data, although the frequency of SI classes varied among populations. In nested ANOVA, the arcsine-transformed values for day 1 self-pollen tubes varied significantly among populations and among plants within populations (table 1). Population means for day 1 self-pollen tubes (untransformed) ranged from 2.1 to 33.8. Variation among plants within populations contributed the most to total variance (67%), while variation among populations was smaller in magnitude (17% of total variance; table 1). Populations included plants of two to four different SI classes (fig. 3). Frequencies of SI classes 3 and 4, which were generally low, were pooled in the χ^2 analysis to avoid bias in the statistic. The χ^2 test strongly rejected the null hypothesis that SI class frequencies were independent of population source ($\chi^2 = 278.9$, $df = 32$, $P < 0.001$), indicating that populations differ significantly in the frequency of SI classes.

Seed Set

Seed set from both self-pollination treatments (autonomous and facilitated) was substantial in all populations (fig. 4), demonstrating the potential for some self-fertilization.

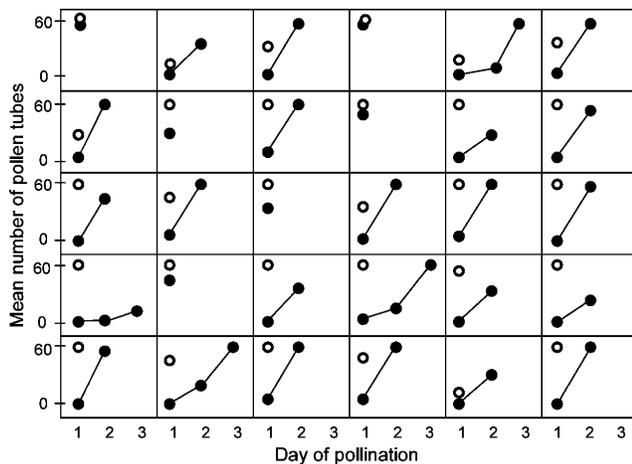


Fig. 2 Mean pollen tube numbers for self- and outcross pollinations in 30 individuals from population 14 (final sample size reduced to 30 as a result of plant mortality). Means were calculated from three replicate flowers per plant and treatment. Open circles = outcross pollinations; closed circles = self pollinations. Self-pollinations of day 2 and day 3 flowers were carried out only in plants that failed to produce >20 self-pollen tubes in day 1 and day 2 flowers, respectively.

Table 1

Nested ANOVA for Self-Pollen Tube Numbers in Day 1 Flowers				
Source of variation	df	MS	F	ω^2
Population	16	5.354	7.91*	16.68
Plant (population)	492	0.677	13.18*	66.96
Error	1009	0.051		16.36

Note. See text for details on data transformation. Populations and plants are treated as random factors. ω^2 = magnitude of effect, or percent of total variance explained (for calculation, see Graham 2001).

* $P < 0.001$.

The effect of pollination treatment on seeds per flower was significant, however (table 2), and post hoc tests showed that outcross seed number was significantly higher than that from facilitated ($P < 0.001$) or autonomous ($P < 0.001$) self-pollination. Seeds per flower in the two self-pollination treatments did not differ ($P = 0.293$), indicating that self seed set was not limited by autonomous deposition of pollen in most populations. The interaction between treatment and population was significant, and inspection of the populations means reveals that the difference between self and outcross seeds per flower was generally greater in populations with high SI indices (fig. 4). When individuals from all populations were combined, we found a weak but significant negative correlation between self-pollen tube number in day 1 flowers and the difference between outcross and autonomous self seeds per flower ($r = -0.236$, $P < 0.001$, $N = 285$).

Relationships between Pollen-Pistil and Floral Morphological Traits

We found highly significant variation among populations and among plants within populations in the three floral traits that were measured (table 3). For corolla lobe length and corolla tube length, variability among population means contributed the most to total variance (table 3). Variation among plants within populations contributed a smaller but substantial component of the total in both traits. For stigma-anther separation, variation within and among plants was comparable in magnitude, while variation among populations contributed considerably less to total variance (table 3). Population SI index was positively and significantly correlated with population means for each of the floral traits (corolla tube length: $r = 0.763$, $P < 0.001$; corolla lobe length: $r = 0.622$, $P = 0.008$; stigma-anther separation: $r = 0.630$, $P = 0.007$; fig. 5). In contrast, when individual plant data were used, we found little evidence for a relationship between the timing of SI (number of self-pollen tubes on day 1) and floral traits. Of the 51 correlations with self-pollen tube number (17 populations \times three floral traits), only four were significant at the $\alpha = 0.05$ level, and only one of these (between self-pollen tubes and corolla lobe length in population 16; $r = -0.632$, $P < 0.001$) was significant after adjusting the α value for multiple tests. The lack of strong correlations between floral and pollen-pistil traits among individuals indicates that the relationships observed at the population level do not reflect genetic correlations caused by linkage or pleiotropy.

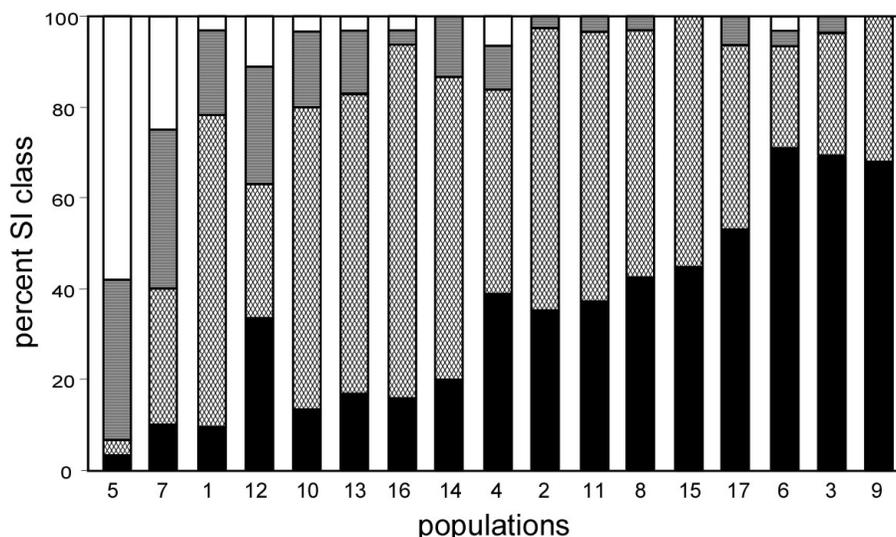


Fig. 3 Percentages of self-incompatibility (SI) classes in 17 study populations. Population SI index is given at top of bars (see text for calculation of SI index). Black bars = class 1 (self-compatible [SC] on day 1); hatched bars = class 2 (SI on day 1, SC on day 2); gray bars = class 3 (SI on days 1 and 2, SC on day 3); open bars = class 4 (SI for days 1–3).

Although each of the mean floral traits was positively related to the SI index, the strength of correlations among floral traits was variable. With respect to population means, corolla lobe length was significantly correlated to stigma-anther separation ($r = 0.737$, $P = 0.001$), but corolla tube length was significantly correlated to neither corolla lobe length ($r = 0.398$, $P = 0.113$; fig. 6) nor stigma-anther separation ($r = 0.344$, $P = 0.177$). The pattern was somewhat congruent when correlations were carried out at the level of individual plants within populations. The single test that was significant at the experiment-wise level was a positive correlation between corolla lobe length and stigma-anther separation (population 2; $r = 0.508$, $P = 0.001$), as were six of the 10 correlations that were significant at the individual test level (r ranged from 0.414 to 0.508; P values ranged from 0.001 to 0.046). Thus, there is some evidence for a genetic correlation between stigma-anther separation and corolla lobe length, but corolla tube length appears to be evolving independently of the other traits.

In Mantel tests, we found that spatial distance was significantly correlated with SI index distance and marginally correlated with floral morphology distances (table 4). However, the correlation coefficient between the matrix for SI index and floral distance remained essentially the same when spatial distance was held constant in a partial Mantel test. This indicates that the relationship between the first two variables cannot be explained by the relationship of each to spatial distance. When we controlled for SI index, the correlation between floral morphology and spatial distance was greatly reduced, indicating that the marginally significant spatial pattern can be attributed largely to the association of floral traits with the SI index. Conversely, the strength of the correlation between SI index and spatial distance was little affected when floral distance was held constant. In summary, there is a significant, though somewhat weak, correspondence between spatial distance and SI index, and the observed rela-

tionship between SI index and floral trait distance appears to be unrelated to geographic similarity.

Mating System Estimates

Estimated outcrossing rates (t) ranged widely among populations (table 5). In both years, the rank ordering of t values was consistent with that of SI indices. Outcrossing rates were higher in all populations in 2004, which raises the possibility that the year-to-year differences resulted from a factor such as variation in pollinator abundance. However, sampling error could also contribute to the variation, especially given that estimates were based on a single locus.

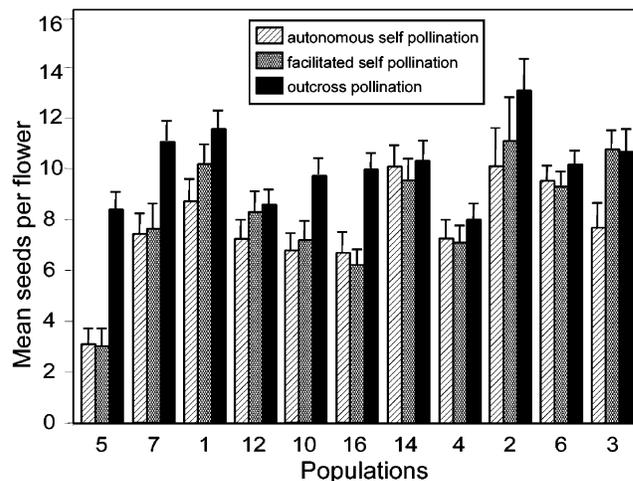


Fig. 4 Mean seeds per flower from three pollination treatments in 11 populations. Populations are arranged from highest (left) to lowest (right) self-incompatibility index (see text for calculation of index). Error bars indicate 1 SE.

Table 2**Mixed Model ANOVA of Seed Number per Flower**

Source of variance	df	MS	F
Pollination treatment	2	446.4	15.77**
Population	10	254.3	8.98**
Treatment \times population	20	28.315	1.89*
Error	861	14.934	

Note. Pollination treatments are cross-pollination, facilitated self-pollination, and autonomous self-pollination.

* $P = 0.01$.

** $P < 0.001$.

Discussion*Variation in the Timing of Self-Compatibility*

Our survey of *Leptosiphon jepsonii* found considerable variation within and among populations in the timing of SC. In all populations sampled, the flowers of some individuals were immediately SC, while others exhibited transient SI, in which self-pollen is rejected until the second or third day after flower opening. Moreover, populations varied significantly in the frequency of these phenotypes, with the proportion of fully SC individuals ranging from 3.2% in population 5 to 71.0% in population 6. The timing of SC is expected to influence the selfing rate of individuals because competing selfing reduces opportunities for outcrossing relative to delayed selfing. Allozyme analyses of three populations indicate a correspondence between the frequency of SI classes and the population outcrossing rate. Although estimates based on single loci must be interpreted with considerable caution, the strong congruence of t with the SI index in both years provides support for the hypothesized relationship.

The differentiation of populations occurs at a markedly fine geographic scale. For instance, note that population 7 and population 9, which are nearly at the two ends of the selfing continuum (fig. 3), are separated by only 6 km (fig. 1). Interestingly, other studies have found substantial variation in mating or breeding systems among populations separated by similarly small distances. For example, populations of *Leavenworthia crassa* occurring within a single square mile differed significantly in outcrossing rates and floral traits (Lyons and Antonovics 1991), and distinct selfing and outcrossing populations of *Clarkia xantiana* were found within an area of just 80 m² (Moore and Lewis 1964). In contrast, however, spatial patterns of mating system variation in other plant taxa often occur at a considerably larger scale. For example, predominantly SI populations of *Trillium kamtschaticum* (Liliaceae) in eastern Hokkaida, Japan, are separated by hundreds of kilometers from most of the SC populations in northern and southern regions (Ohara et al. 1996). Similarly, selfing and outcrossing populations of *Arenaria uniflora* generally occur in geographically distinct regions of the south-eastern United States (Wyatt 1984).

This study does not provide estimates of heritability, but several lines of evidence suggest a strong genetic contribution to variation in the timing of SC. First, environmental and developmental factors were kept constant, to a large extent. To control for developmental factors such as plant age or pres-

ence of fruits that have been shown to influence SI in other species (Vogler et al. 1998), we carried out all experimental pollinations on the first several flowers to open before any fruit set occurred. Although all populations were not assayed simultaneously, ambient temperature was maintained within a fairly narrow range in the growth room, which minimized the potential for temperature effects on SI (Wilkins and Thorogood 1992; Jakobsen and Martens 1994). Second, in a study now in progress, we have developed inbred lines that differ dramatically in self-pollen tube number for populations 1, 4, and 6 (C. Goodwillie, unpublished data). Third, growth room and field experiments in a previous study yielded remarkably similar frequencies of fully SC individuals for a population at Wantrup Reserve (population 16 in this study; Goodwillie et al. 2004). However, in a minority of plants, pollen tube numbers varied considerably among replicate flowers, indicating that nongenetic factors also contribute to this variation.

Relationship between Pollen-Pistil Interactions and Floral Morphology

The population SI index was significantly related to population means for each of the three floral traits measured. Based on the lack of correlation among individual plants, these did not appear to be caused by genetic linkage or pleiotropy. Instead, the population-level correlations suggest that fine-scale local adaptation of, or in response to, the selfing rate is occurring in *L. jepsonii*. A relationship between floral size and mating systems has been documented in comparisons among plant species (Cruden and Lyon 1985; Ritland and Ritland 1989; Affre and Thompson 1998; Goodwillie 1999; Armbruster et al. 2002) and, to a lesser extent, among populations within species (Wyatt 1984; Lyons and Antonovics 1991). This has been interpreted in the context of resource allocation; if in selfing populations resources required for attractive structures can be diverted to other fitness-related functions such as seed maturation, reduction in corolla size will be selectively favored (Cruden and Lyon 1985;

Table 3**Nested ANOVA for Three Floral Traits**

Trait/source of variance	df	MS	F	ω^2
Corolla lobe length:				
Population	16	10.425	28.70*	42.33
Plant (population)	502	0.363	7.72*	39.88
Error	1037	0.047		17.79
Corolla tube length:				
Population	16	1989.531	42.08*	53.84
Plant (population)	502	47.282	11.50*	35.90
Error	1037	4.113		10.26
Stigma-anther separation:				
Population	16	2.125	12.65*	20.62
Plant (population)	502	0.169	4.09*	40.48
Error	1037	0.041		38.90

Note. Populations and individuals are treated as random factors. ω^2 = magnitude of effect, or percent of total variance explained (for calculation, see Graham 2001).

* $P < 0.001$.

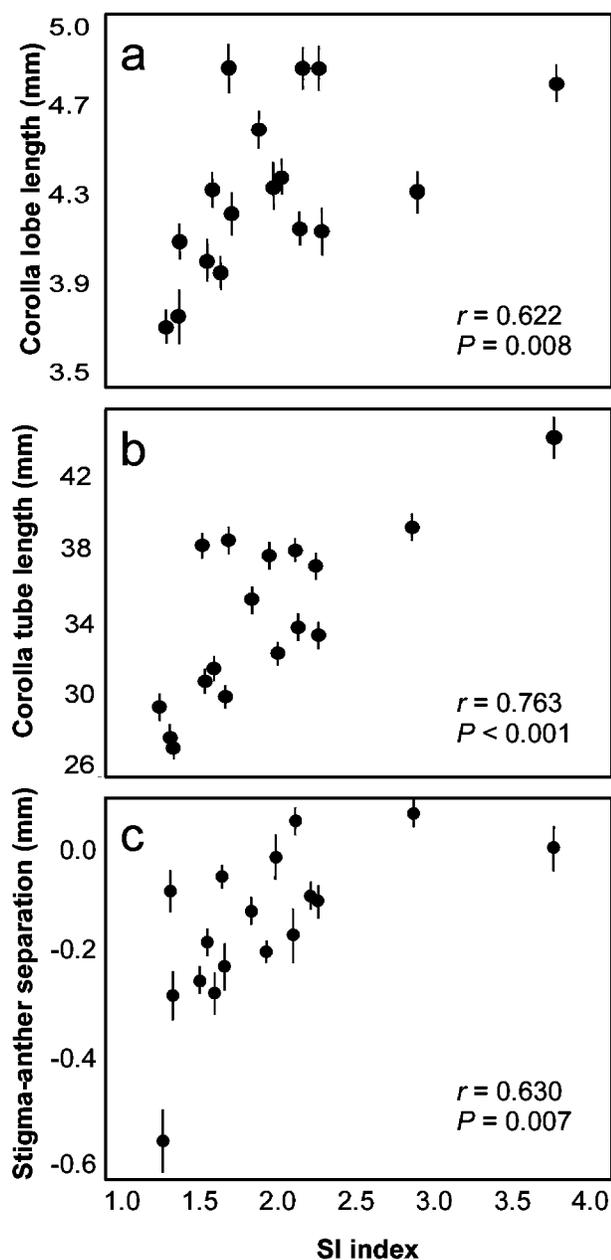


Fig. 5 Scattergrams of mean floral trait values versus self-incompatibility index for 17 populations: a, corolla lobe length; b, corolla tube length; c, stigma-anther separation. Error bars indicate 1 SE for floral traits.

Charlesworth and Charlesworth 1987). We found significant variation among populations in two floral size traits, and population means for each was significantly correlated with the population SI index in the direction predicted by theory.

Spatial overlap of stigma and anthers, which is associated with high selfing rates in other plant taxa (Lyons and Antonovics 1991; Dole 1992; Belaousoff and Shore 1995; Motten and Stone 2000), was observed in populations of *L. jepsonii* with low SI indices. While shifts in corolla size may reflect adaptation in response to a change in the selfing

rate, stigma-anther position is expected to be a primary determinant of the selfing rate in SC species because it affects rates of self-pollen deposition. Selection to increase or decrease the rate of selfing may be acting concurrently on the timing of SI and on stigma-anther position in *L. jepsonii*. Positive correlations between stigma-anther separation and corolla tube length suggest genetic covariation of the traits and the possibility that evolution in one may be affected by selection on the other. In addition, the arrangement of stigma and anthers can affect the placement and delivery of pollen by vectors and can therefore influence outcross success. Thus, the potential selective factors acting on stigma-anther separation are complex and require further study.

A possible complicating factor demands consideration. Floral and pollen-pistil traits were measured in plants grown from field-collected seeds for which the history of inbreeding is variable and unknown. Inbreeding depression for floral traits could cause a reduction in flower size in more SC populations, as has been hypothesized in a study of floral size variation in *Crepis tectorum* (Andersson 1996). It seems unlikely, however, that this factor contributes substantially to the observed correlation. First, if inbreeding depression is largely responsible for reduced flower size, one might expect to find some correlation between floral size and SC at the level of individuals, and that relationship was not found. Second, in a previous experimental study of inbreeding depression of *L. jepsonii* (Goodwillie 2000), the magnitude of inbreeding depression for flower size in population 16 was found to be considerably less than the differences observed among populations in this study (C. Goodwillie, unpublished floral morphology data). For example, mean corolla lobe length in selfed offspring (4.36 mm) was only 1.2% less than that in outcrossed offspring (4.41 mm), as compared with a 21.1% difference between the smallest (3.73 mm, population 9) and the largest (4.77 mm, population 10) mean corolla lobe length observed in this study.

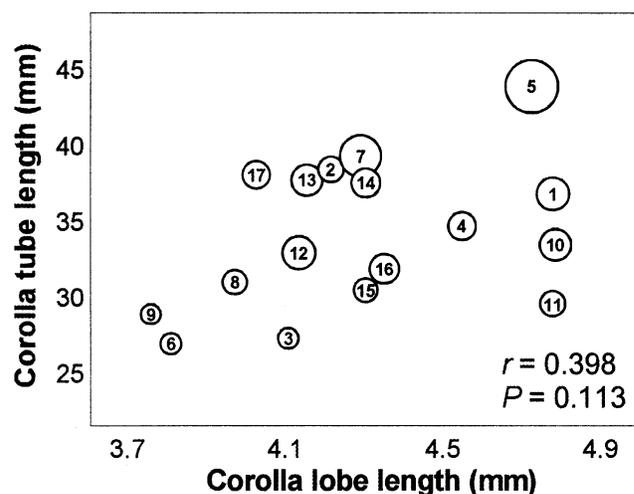


Fig. 6 Scattergram of mean corolla tube length versus corolla lobe length for 17 populations. Size of circle is proportional to value of self-incompatibility index (range = 1.35–3.56). Population numbers are indicated inside circles.

Table 4**Results of Simple and Partial Mantel Tests**

Distance matrices compared	Distance matrix held constant	Correlation coefficient	<i>P</i>
SI and floral	...	0.584	0.001
SI and floral	Spatial	0.560	0.001
Floral and spatial	...	0.198	0.065
Floral and spatial	SI	-0.0143	0.864
SI and spatial	...	0.358	0.027
SI and spatial	Floral	0.305	0.034

Note. *P* values obtained from permutation tests with 999 iterations. SI = self-incompatibility.

Evolution of Mating Systems

What factors are driving the evolutionary dynamics at this interface between SI and self-fertilization? Our data provide a starting point for understanding. The presence of different SI classes in *L. jepsonii* can be viewed as variation in the mode of selfing, where transient SI confers selfing that is delayed until after opportunities for outcrossing and full SC promotes competing selfing.

Selection for reproductive assurance has been invoked as a driver in the evolution of self-fertilization (Stebbins 1957; Baker 1959). Whether it plays a role in the evolution of the timing of SC in *L. jepsonii* depends in part on the relative amount of seed set provided by delayed and competing selfing in the absence of pollinators. Although seed set from self-pollination (both autonomous and facilitated) was generally substantial in *L. jepsonii*, it was significantly lower overall than that from cross pollination, and the difference was greatest in populations with a low frequency of early SC (competing selfing) phenotypes (fig. 4). As expected, self seed set was quite low in population 5, in which pollen tube data indicate that many plants are fully SI. Surprisingly, however, the difference between outcross and self seed set was also considerable in some populations comprising mostly transient SI plants (e.g., populations 10 and 16; fig. 4). We also observed a significant relationship between early self-pollen tube growth and autonomous seed set at the level of individuals. Taken together, these results indicate that the potential for reproductive assurance provided by transient SI, while substantial, is less than that conferred by early SC. Reduced selfed seed set in plants with transient SI could reflect the per-

sistence of some degree of partial SI in older flowers. Alternatively, it could indicate a cost of delaying fertilization such that there is insufficient time for pollen tube growth before flowers or ovaries senesce. Differences observed in the seed set of selfed and outcrossed flowers could also result from early inbreeding depression. The pattern seen among populations of *L. jepsonii* is consistent with an expectation of higher inbreeding depression in more outcrossing populations when deleterious recessive alleles are purged with selfing (Lande and Schemske 1985; Husband and Schemske 1993). In a preliminary study of plants from population 16 (with an intermediate SI index of 2.0), dissections of selfed and outcrossed fruits show a higher proportion of both unfertilized ovules and aborted seeds in selfed relative to outcrossed fruits (C. Goodwillie, unpublished data), indicating that both pre- and postfertilization events may account for differences in self and outcross seed set. Thus, differences among population might reflect variation in pollinator abundance that selects on the timing of selfing for reproductive assurance. In addition, variation in the magnitude of inbreeding depression may exert differential selection on the selfing rate among populations.

Our data provide evidence for a spatial pattern in mating system variation, which may yield insights into the evolutionary processes acting on this variation. Distances among populations in SI index were significantly related to spatial distance, with more highly selfing populations often found at the southeastern end of the distribution (figs. 1, 3). For instance, population 5, at the northwestern end of the species distribution, has the highest SI index, with the majority of individuals appearing to be fully SI. In contrast, the eight most highly SC populations include six that are found in the southern half of the distribution. Although population sites occur within a relatively small geographical area and occupy apparently similar oak woodland habitats, the possibility exists that this pattern may indicate an underlying ecological factor that influences selection on SI variation, such as a gradient in pollinator abundance.

While spatial distances among populations are correlated with SI distance and marginally correlated with floral distance, the results of a partial Mantel test indicate that the relationship of floral traits to SI is independent of the geographical pattern. This indicates that associations between the timing of SI and floral morphology have arisen independently in different populations, strengthening the argument for an

Table 5**Mating System Parameters for Three Populations Spanning the Range of SI Indices**

	Population 6	Population 16	Population 7
SI index	1.42	2.00	2.78
Mating system estimates:			
2003	<i>t</i> = 0.006 (0.007) <i>F</i> = 0.562 (0.153) 44 families, 591 progeny	<i>t</i> = 0.296 (0.055) <i>F</i> = 0.141 (0.151) 47 families, 630 progeny	<i>t</i> = 0.679 (0.146) <i>F</i> = 0.029 (0.164) 42 families, 601 progeny
2004	<i>t</i> = 0.125 (0.096) <i>F</i> = 0.489 (0.217) 50 families, 785 progeny	<i>t</i> = 0.504 (0.107) <i>F</i> = 0.218 (0.192) 45 families, 633 progeny	<i>t</i> = 0.699 (0.120) <i>F</i> = 0.131 (0.175) 47 families, 709 progeny

Note. Outcrossing rates (*t*) and fixation indices (*F*) estimated from single locus allozyme analysis. Standard errors based on 1000 bootstrap replicates with maternal family as the resampled unit. SI = self-incompatibility.

adaptive process. Moreover, although SI index was positively correlated with population means for both corolla tube and lobe length, the two trait means were not significantly related to each other, indicating that populations with similar SI indices differ in their floral allometry. For instance, if we compare populations 11 and 17 with similar and moderately low SI indices (fig. 6), we find that the reductions in corolla size occur in different dimensions; population 11 has short tubes and long corolla lobes, and the reverse is true for population 17. An intriguing possibility is that selection is acting on different standing mutations and following a different sequence of adaptive steps as populations converge on similar flower size.

Conclusions

The genus *Leptosiphon* contains species that are SI, large-flowered, and obligately outcrossing and others that are SC, small-flowered, and highly selfing. The finding in *L. jepsonii* of considerable variation in both floral traits and SI indicates that the mating system of this intermediate species is evolutionarily dynamic, providing an opportunity to study the evolutionary processes associated with this transition. Our phenotypic data indicate that coordinated adaptation of floral

size, spatial arrangement of stigma and anthers, and pollen-stil traits has occurred in populations of this species. A more complete understanding of the selective factors acting on variation in *L. jepsonii* and the stability of its intermediate mating system will require detailed studies that address pollinator visitation, inbreeding depression, reproductive assurance, heritability of traits, and the evolutionary relationships among populations.

Acknowledgments

We thank M. C. Knight, J. Kornegay, K. Partis, G. Patel, and J. Weber for assistance with experimental pollinations and microscopy; S. Gardiner and B. Neel for running allozyme gels; P. Marek for assistance with figure 1; C. Jolls for generously sharing growth chamber space; and J. Callizo of the Napa County Land Trust, M. Gillogly of the California Academy of Sciences, and Juliana Vineyards for access to population sites. The manuscript was improved by the valuable comments of two anonymous reviewers. This study was supported by an East Carolina University Research and Creativity Award and National Science Foundation grant DEB-0212903 to C. Goodwillie.

Literature Cited

- Affre L, JD Thompson 1998 Floral trait variation in four *Cyclamen* (Primulaceae) species. *Plant Syst Evol* 212:279–293.
- Andersson S 1996 Floral reduction in *Crepis tectorum* (Asteraceae): trade-offs and dominance relationships. *Biol J Linn Soc* 57:59–68.
- Armbruster WS, CPH Mulder, BG Baldwin, S Kalisz, B Wessa, H Nute 2002 Comparative analysis of late floral development and mating-system evolution in tribe Collinsieae (Scrophulariaceae SL). *Am J Bot* 89:37–49.
- Arroyo MT 1975 Electrophoretic studies of genetic variation in natural populations of allogamous *Limnanthes alba* and autogamous *Limnanthes floccosa* (Limnanthaceae). *Heredity* 35:153–164.
- Baker HG 1959 Reproductive methods as factors in speciation in flowering plants. *Cold Spring Harbor Symp Quant Biol* 24:177–190.
- Becerra J, DG Lloyd 1992 Competition-dependent abscission of self-pollinated flowers of *Phormium tenax* (Agavaceae): a second action of self-incompatibility at the whole-flower level? *Evolution* 46:458–469.
- Belaoussoff S, JS Shore 1995 Floral correlates and fitness consequences of mating system variation in *Turnera ulmifolia*. *Evolution* 49:545–556.
- Charlesworth D, B Charlesworth 1987 The effect of investment in attractive structures on allocation to male and female functions in plants. *Evolution* 4:948–968.
- Cruden RW, DL Lyon 1985 Patterns of biomass allocation to male and female functions in plants with different mating systems. *Oecologia* 66:299–306.
- Culley TM 2002 Reproductive biology and delayed selfing in *Viola pubescens* (Violaceae), an understory herb with chasmogamous and cleistogamous flowers. *Int J Plant Sci* 163:113–122.
- de Nettancourt D 1977 Incompatibility in angiosperms. Springer, New York.
- Dole JA 1992 Reproductive assurance mechanisms in three taxa of the *Mimulus guttatus* complex (Scrophulariaceae). *Am J Bot* 79:650–659.
- Ennos RA 1981 Quantitative studies of the mating system in two sympatric species of *Ipomoea* (Convolvulaceae). *Genetica* 57:93–98.
- Goodwillie C 1999 Multiple origins of self-compatibility in *Linanthus* section *Leptosiphon* (Polemoniaceae): phylogenetic evidence from internal-transcribed-spacer sequence data. *Evolution* 53:1387–1395.
- 2000 Inbreeding depression and mating systems in two species of *Linanthus* (Polemoniaceae). *Heredity* 84:283–293.
- Goodwillie C, KL Partis, JW West 2004 Transient self-incompatibility confers delayed selfing in *Leptosiphon jepsonii* (Polemoniaceae). *Int J Plant Sci* 165:387–394.
- Graham MH 2001 Statistical significance versus fit: estimating the importance of individual factors in ecology analysis of variance. *Oikos* 93:505–513.
- Harder LD, WG Wilson 1998 A clarification of pollen discounting and its joint effects with inbreeding depression on mating system evolution. *Am Nat* 152:684–695.
- Herlihy CR, CG Eckert 2004 Experimental dissection of inbreeding and its adaptive significance in a flowering plant, *Aquilegia canadensis* (Ranunculaceae). *Evolution* 58:2693–2703.
- Holtsford TP, NC Ellstrand 1989 Variation in the outcrossing rate and population genetic structure of *Clarkia tembloriensis* (Onagraceae). *Theor Appl Genet* 78:480–488.
- Husband BCH, DW Schemske 1993 Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50:54–70.
- Jakobsen HB, H Martens 1994 Influence of temperature and aging of ovules and pollen on reproductive success in *Trifolium repens*. *Ann Bot* 74:493–501.
- Kalisz S, D Vogler, B Fails, M Finer, E Shepard, T Herman, R Gonzales 1999 The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). *Am J Bot* 86:1239–1247.
- Karron J, NN Thumser, R Tucker, AJ Hennessee 1995 The influence of population density on outcrossing rates in *Mimulus ringens*. *Heredity* 75:175–180.

- Lande R, DW Schemske 1985 The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39: 24–40.
- Leclerc-Potvin C, K Ritland 1994 Modes of self-fertilization in *Mimulus guttatus* (Scrophulariaceae): a field experiment. *Am J Bot* 81:199–205.
- Lipow SR, SB Broyles, R Wyatt 1999 Population differences in self-fertility in the “self-incompatible” milkweed *Asclepias exaltata* (Asclepiadaceae). *Am J Bot* 86:1114–1120.
- Lloyd DG 1965 Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). *Contrib Gray Herb Harv Univ* 195:3–134.
- 1979 Some reproductive factors affecting the selection of self-fertilization in plants. *Am Nat* 113:67–97.
- 1992 Self-fertilization and cross-fertilization in plants. II. The selection of self-fertilization. *Int J Plant Sci* 153:370–380.
- Lloyd DG, DJ Schoen 1992 Self-fertilization and cross-fertilization in plants. I. Functional dimensions. *Int J Plant Sci* 153:358–369.
- Lu YQ 2000 Effects of density on mixed mating systems and reproduction in natural populations of *Impatiens capensis*. *Int J Plant Sci* 161:671–681.
- Lyons EE, J Antonovics 1991 Breeding system evolution in *Leavenworthia*: breeding system variation and reproductive success in natural populations of *Leavenworthia crassa* (Cruciferae). *Am J Bot* 78:270–287.
- McClure BA, F Cruz-Garcia, B Beecher, W Sulaman 2000 Factors affecting inter- and intra-specific pollen rejection in *Nicotiana*. *Ann Bot* 85:113–123.
- Moore DM, H Lewis 1964 The evolution of self-pollination in *Clarkia xantiana*. *Evolution* 19:104–114.
- Motten AF, J Antonovics 1992 Determinants of outcrossing rate in a predominantly self-fertilizing weed, *Datura stramonium* (Solanaceae). *Am J Bot* 79:419–427.
- Motten AF, JL Stone 2000 Heritability of stigma position and the effect of stigma-anther separation on outcrossing in a predominantly self-fertilizing weed, *Datura stramonium* (Solanaceae). *Am J Bot* 87:339–347.
- Nasrallah M, MK Kandasamy, JB Nasrallah 1992 A genetically defined *trans*-acting locus regulates *S*-locus function in *Brassica*. *Plant J* 2:497–506.
- Ohara M, H Takeda, Y Ohno, Y Shimamoto 1996 Variations in the breeding system and the population genetic structure of *Trillium kamtschaticum* (Liliaceae). *Heredity* 76:476–484.
- Ornduff R 1969 Reproductive biology in relation to systematics. *Taxon* 18:121–133.
- Rice WR 1989 Analyzing tables of statistical tests. *Evolution* 43: 223–225.
- Rick CM, JF Fobes, SD Tanksley 1979 Evolution of mating system in *Lycopersicon hirsutum* as deduced from genetic variation in electrophoretic and morphological characters. *Plant Syst Evol* 132:270–298.
- Ritland C, K Ritland 1989 Variation of sex allocation among eight taxa of the *Mimulus guttatus* species complex (Scrophulariaceae). *Am J Bot* 76:1731–1739.
- Rosenberg MS 2001 PASSAGE: pattern analysis, spatial statistics, and geographic exegesis. Version 1.1.2.3. Department of Biology, Arizona State University, Tempe.
- Routley MB, K Mavraganis, CG Eckert 1999 Effect of population size on the mating system in a self-compatible, autogamous plant, *Aquilegia canadensis* (Ranunculaceae). *Heredity* 82:518–528.
- Schemske DW, C Goodwillie 1996 Morphological and reproductive characteristics of *Linanthus jepsonii* (Polemoniaceae), a newly described, geographically restricted species from Northern California. *Madroño* 43:453–463.
- Schoen DJ 1982 The breeding system of *Gilia achilleifolia*: variation in floral characteristics and outcrossing rate. *Evolution* 36:352–360.
- Smouse PE, JC Long, RR Sokal 1986 Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Syst Zool* 35:627–632.
- Solbrig OT, RC Rollins 1977 The evolution of autogamy in species of the mustard genus *Leavenworthia*. *Evolution* 31:265–281.
- SPSS 2003 SPSS. Version 12. SPSS, Chicago.
- Stebbins GL 1950 Variation and evolution in plants. Columbia University Press, New York.
- 1957 Self-fertilization and population variability in higher plants. *Am Nat* 41:337–354.
- 1974 Flowering plants: evolution above the species level. Belknap, Cambridge, MA.
- Stephenson AG, JA Winsor, TE Richardson, A Singh, T-H Kao 1992 Effects of style age on the performance of self and cross pollen in *Campanula rapunculoides*. Pages 77–121 in E Ottaviano, DL Mulcahy, M Sari Gorla, G Bergamini Mulcahy, eds. *Angiosperm pollen and ovules*. Springer, New York.
- Stone JL 2002 Molecular mechanisms underlying the breakdown of gametophytic self-incompatibility. *Q Rev Biol* 77:17–32.
- Vogler DW, C Das, AG Stephenson 1998 Phenotypic plasticity in the expression of self-incompatibility in *Campanula rapunculoides*. *Heredity* 81:546–555.
- Vogler DW, AG Stephenson 2001 The potential for mixed mating in a self-incompatible plant. *Int J Plant Sci* 162:801–805.
- Werth CR 1985 Implementing an isozyme lab at a field station. *Va J Sci* 36:53–76.
- Wilkins PW, D Thorogood 1992 Breakdown of self-incompatibility in perennial ryegrass at high-temperature and its uses in breeding. *Euphytica* 64:65–69.
- Wyatt R 1984 The evolution of self-pollination in granite outcrop species of *Arenaria* (Caryophyllaceae). I. Morphological correlates. *Evolution* 38:804–816.