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BREEDING SYSTEM EVOLUTION IN LEAVENWORTHIA: BREEDING SYSTEM VARIATION AND REPRODUCTIVE SUCCESS IN NATURAL POPULATIONS OF LEAVENWORTHIA CRASSA (CRUCIFERAE)

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Populations of Leavenworthia crassa (Cruciferae) studied for 3 years exhibited among-and within-population genetic variation for a suite of floral and reproductive traits (flower width, petal length, anther position, ability to set seeds in the absence of pollinators, time to first flowering) associated with breeding system. We used electrophoretic markers to show that a population with small, monomorphically colored flowers with introrse anthers had a significantly lower outcrossing rate (t = 0.03) than did a population with larger, polymorphically colored flowers with extrorse anthers (t = 0.33). In the more-outcrossing population the correlation between higher maternal plant outcrossing rate and the suite of six traits approached significance (P < 0.067), with greater petal size, greater flower width, and reduced ability to set seeds in the absence of pollinators contributing significantly. Plants in selfing populations had a generally higher reproductive success, with a higher number of flowers per plant, a smaller proportion of unfertilized ovules, a smaller proportion of fertilized ovules aborted, a higher rate of fruit set, and overall a larger number of seeds matured than did plants from the more outcrossing populations. Pollen limitation did not appear to account for lower reproductive success in outcrossing populations. Resource limitation did not differ substantially between populations. However, within-ovary patterns of fertilization, abortion, and seed weight were significantly less random in outcrossing populations than in selfing populations, suggesting that differential gamete and embryo success may be responsible for lower reproductive success in outcrossing populations.

Because plant breeding systems determine their own transmission into the next generation as well as the mode of transmission for all other genes in a plant, they have the potential to profoundly influence the amount and structure of genetic variation within a population. Until the last 5–10 years, many studies of plant breeding system evolution were static, correlational, or descriptive (Wyatt, 1983; Uyeno-

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yama and Antonovics, 1987). The recent heightened interest in the evolution of complex plant breeding systems has been marked by an increase in experimental approaches and by a more substantial theoretical foundation on which to construct testable hypotheses. Such a framework is now emerging, with numerous recent papers outlining theoretical approaches to the study of plant sexual systems (Bengtsson, 1978; Charlesworth and Charlesworth, 1979; Lloyd, 1979; Schemske and Lande, 1985; Uyenoyama and Antonovics, 1987; Holsinger, 1988).

Experimental work has often lagged behind theoretical advances. This is, in part, due to the difficulty in getting field estimates for factors likely to influence breeding system evolution such as genetic and environmental variation in floral traits and outcrossing rates, reproductive success under different breeding systems, and levels of inbreeding and outbreeding depression, biparental inbreeding, and sibling competition. While each of these factors has been studied in one to many species, for only a few plant species do we have even a fraction of this information from natural populations. The numerous studies on Impatiens
capensis by Waller, Schemske, Schmitt, and colleagues are exceptions (e.g., Waller and Knight, 1989 and citations therein).

This is the first in a series of papers describing components of breeding system evolution in the genus Leavenworthia. This genus has been the subject of a number of classic breeding system studies (Rollins, 1963; Lloyd, 1965; Solbrig, 1972; Solbrig and Rollins, 1977). Rollins (1963) suggested that the genus is especially well suited for such studies because there is wide variation within and among species in floral traits (flower color, size, anther position) and because the habitats in which these species grow are ecologically simple and relatively uniform, allowing an examination of evolutionary patterns where environmental differences are minimal.

Rollins (1963) and Lloyd (1965) took a biodynamic approach to variation in floral traits such as flower color, flower width, petal length, and anther position. They used population means to assign populations to races or species. In this paper we expand those studies of floral traits in *L. crassa* by examining the genetic component of variation in floral traits both within and among populations. We examine two sets of populations, one with traits associated with predominant selfing (small, monomorphically colored flowers with introrse anthers) and the other with traits associated with predominant outcrossing (larger, polymorphically colored flowers with extrorse anthers).

Solbrig (1972) and Solbrig and Rollins (1977) examined electrophoretic variation in *L. crassa* and found that small-flowered populations had fewer polymorphic loci and more nonsegregating families than did populations with larger flowers. We extend the analysis of electrophoretic variation by estimating outcrossing rates and by attempting to correlate outcrossing rates with floral traits, both across populations and within populations.

For over 20 populations of *L. crassa*, Lloyd (1965) examined several components of reproductive success (ovule number, pollen tube growth, seed set in the greenhouse, seed weight, and number of flowers). We describe a 3-year study of reproductive success in selfing and more outcrossing populations that extends Lloyd’s work on this species by: 1) examining reproduction in more detail, breaking it down into different stages of reproduction during which different selective forces might act; 2) comparing reproductive success across similar populations in order to separate environmental vs. breeding system contributions to reproductive success; and 3) examining factors such as pollen limitation, resource limitation, and differential male and female reproductive success, that could constrain reproduction in these natural populations. The patterns of reproduction that we document will also serve as a baseline for additional experimental studies on reproductive success under different breeding systems (Loyds, 1986).

We here address the following questions:
1) Is there within-populations and/or among-population genetic variation for floral traits likely to affect outcrossing rate?
2) Do populations with those floral traits associated with selfing have a lower outcrossing rate than populations with floral traits associated more with outcrossing? Is there a correlation within populations between the outcrossing rate of individual plants and their floral traits?
3) Do populations with different floral traits and mating systems differ in reproductive success? Are differences in reproductive success attributable to differences in breeding system and/or to differences in habitat?
4) Are factors that influence reproductive success, such as pollen limitation, resource limitation, and different patterns of male and female success, commensurate in selfing vs. more outcrossing populations?

**MATERIALS AND METHODS**

*Leavenworthia crassa* (Cruciferae) Rollins is a winter annual narrowly endemic to an area roughly 13 miles in diameter in north central Alabama (Rollins, 1963). Lloyd (1965) described populations within a 1-square-mile area that exhibited traits associated with widely divergent breeding systems. We chose populations that showed easily recognizable traits associated with either a more outcrossing breeding system (large strongly scented flowers with two color morphs and extrorse anthers) or with a more selfing breeding system (small monomorphically colored flowers with little scent and introrse anthers) (Lloyd, 1965).

The putative selfing populations were Populations 1 and 2 (Lloyd’s race c12, Population 739 and race c13, Population 745, respectively), and the putative outcrossing populations were Populations 4, 5, and 7 (Lloyd’s race c5 [1965]). All five populations were located within a 1-square-mile area in Massey, Alabama.

**Variation in plant mating system traits** — To estimate among and within population variation for several floral and reproductive traits we collected seeds as 16–20 maternal families from both a putative selfing population (Population 1) and from a putative outcrossing pop-
ulation (Population 5) in the spring of 1983. We grew three to five offspring from each of these families in a randomized block design in a pollinator-free growth chamber (12 hour day-length, day/night temperature 20/5 C) at the Duke University Phytotron and measured date of first flowering and total flower number, and for five to ten flowers and subsequent fruits, we also measured petal length, flower width, anther position, ovule number, seed number, and individual seed weight. We scored anther position as a continuous character, modifying the notation of Rollins (1963), assigning a value of 1 to introorse anthers, 2 to intermediate anthers, and 3 to extrorse anthers (Fig. 1).

We used a nested analysis of variance, with a planting block effect included, to determine population and family effects on floral and reproductive traits. All standard statistical analyses were conducted using the Statistical Analysis System (SAS Institute, 1985) installed at the Triangle University Computation Center and at Amherst College.

We conducted a smaller survey of floral traits in two additional populations, Population 2 (small flowered, putatively selfing plants) and Population 4 (large flowered, putatively outcrossing plants). We sampled seeds as above and grew the plants in the Phytotron, randomized in among the plants from Populations 1 and 5. We measured flower width, petal length, and anther position and statistically compared all four populations for these characters.

In their earlier work on L. crassa, Lloyd (1965) and Solbrig and Rollins (1977) reported breeding system attributes of these same populations and attributed the evolution of these differences to habitat variation across the populations. We examined whether there had been change in two floral traits, petal length, and anther position, in the 20 years since Lloyd’s work. Because flower (and plant) size tends to be somewhat larger in Phytotron-grown plants than in field-grown plants, we compared measurements that we made on field-grown plants with Lloyd’s field measurements.

Solbrig and Rollins (1977) suggested that differences in moisture regime might have been responsible for the evolution of selfing in previously outcrossing lineages in this species. The cedar glades in which these winter annuals grow have very shallow soils overlying horizontally bedded limestone. The precipitation tends to be very seasonal, with wet weather concentrated in the winter and early spring followed by very dry conditions in the late spring and summer. In early May of 1984, 1 week after the previous rainfall, we took soil samples from within 4 inches of each of 50 plants in each population. We dried the soil and estimated percent soil moisture for each sample. We also measured soil depth at nine points within 6 inches of each plant and used the average around each plant for analysis. We determined population elevations from United States Geological Survey maps.

Outcrossing estimates—We used electrophoretic polymorphisms to estimate the outcrossing rate in one of the populations with traits associated with outcrossing (Population 5) and in one with selfing traits (Population 1). In the spring of 1983 we collected 20–35 flowering plants from each population and placed them in a screened enclosure at the Duke University Greenhouses. We then hand-selfed several of the remaining flowers on each plant in order to get selfed seed for estimating maternal genotypes. When the fruits were mature, we collected seeds from the hand-selfs and from fruits that had been naturally pollinated in the field in Alabama. From the latter we sampled
one to two seeds from as many fruits as possible to minimize correlated outcrossing events within fruits (Schoen and Clegg, 1984). We germinated the seeds, grew the plants to the small rosette stage, harvested leaf tissue, and froze it at −70°C.

We assayed polymorphism using starch gel electrophoresis with the buffers and enzyme stains outlined in Cardy, Stuber, and Goodman (1981) and Meagher (1986). Approximately 30 loci were initially surveyed for each population, but no attempt was made to estimate relative levels of polymorphism at all of those loci. Rather, we identified up to five polymorphic loci that could be unambiguously scored for each population. For three to six selfed progeny and five to 40 naturally pollinated progeny representing four to nine fruits from each maternal plant collected, we assayed four enzymes in the more outcrossing population (five loci: esterase-1 [EST-1], esterase-2 [EST-2], phosphoglucone isomerase [PGI], phosphoglucosidase [PGM], and acid phosphatase [ACP]), and the only three polymorphic enzymes we could find in the selfing population (esterase-1 [EST-1], glutamate oxaloacetate transaminase [GOT], and acid phosphatase [ACP]).

We first estimated maternal genotypes from the three to six selfed progeny. With four such progeny, eight maternal alleles are sampled for each locus, and the probability of detecting a heterozygote at a locus is very high, 1 − (0.5^N) or 0.992. Even if only three selfed progeny are sampled, the probability of detecting a heterozygote is very high (0.969).

We estimated pollen and ovule gene frequencies and outcrossing rates for the two populations using a multilocus algorithm developed by Ritland and Jain (1981). We calculated variances around these estimates of population outcrossing rate using an updated version of this program (Ritland, personal communication), which employs the Newton-Raphson method.

The Ritland program also allowed us to estimate outcrossing rates for maternal families, many of which were the same families used in our analysis of variation in floral and reproductive traits. We next determined the relationship between the outcrossing rate realized by individual maternal plants and their floral traits. Because few flowers remained on maternal plants when we sampled them from the field, we used progeny means for each family, derived from offspring grown in the Phytotron, as a measure of floral traits of maternal parents. We therefore regressed maternal outcrossing rate on family measures of floral traits.

**Reproduction at the field sites—Patterns of natural reproduction**—We monitored reproduction in the field over 3 years. Shortly after germination in late fall 1983, 1984, and 1985 we marked 30–50 randomly chosen individual plants in each of two putative selfing (Populations 1 and 2) and two putative outcrossing field populations (Populations 4 and 7; Population 5 could not be used because it was decimated in the summer of 1983 by a road grading operation). In 1986, Populations 2 and 7 were again monitored. In the spring of 1984, 1985, and 1986 we marked and followed an additional set of ten to 20 randomly chosen large plants (> 15 flower buds in early spring) in each population. In 1986 we also chose at random an additional five to ten plants with the less frequent yellow flower color. In the spring of each year we labeled each flower on each plant with a numbered paper strip and recorded the day each flower opened.

For approximately half of these plants we allowed all of the flowers to be naturally pollinated. The remainder of the plants were used for an experiment on the effects of imposing different breeding systems (Lyons, 1986).

At the end of the fruiting season we collected all of the fruits from marked flowers, counted and weighed (in 1985 and 1986) all seeds, and noted their positions within the fruits. We also recorded the number and position of unfertilized ovules and aborted seeds. The latter appear as unenlarged transparent masses of tissue still connected to the septum wall. We were able to trace the fate of all ovules initiated within an ovary; we found that the number of ovules in an ovary removed immediately after flowering matched very closely the number of ovules in an adjacent flower that was examined after pollination and seed development (Lyons, 1986). Seeds that weighed less than 0.3 mg were never observed to germinate during any of several greenhouse studies. Therefore, any ovule that was enlarged, darkened, and weighed less than 0.3 mg was classified as aborted. We also weighed individual mature seeds.

For each field plant we calculated the mean across all flowers for 1) the proportion of total ovules that were not fertilized, 2) the proportion of fertilized ovules that aborted, 3) the proportion of ovules that matured into seeds, 4) the number of seeds per fruit, and 5) the mean individual seed weight per fruit. The arcsine square root transform was used on the first three variables in all analyses. Because the number of fertilized ovules, aborted seeds, and mature seeds sum to the total number of ovules, the results for these variables will not be in-
dependent and so must be interpreted with caution.

We also estimated for each plant in 1985 and 1986 the proportion of flowers that matured fruit. We did not include in this calculation any flower for which a tag was not recovered; a spring tornado in 1984 washed many fruits and/or tags away, making the data from 1984 unsuitable for this analysis.

In 1986 within the outcrossing population we also tested the effect of the two flower colors on fruit set, fertilization, abortion, and seed set.

The 3 years in which we studied these populations were marked by great meteorological variation. We therefore analyzed the data both by year and summed over all years. Within each year, we used an analysis of covariance, with date as the covariate, to test whether reproductive patterns varied across the length of the flowering season. For only seed weight and fruit set was the date covariate significant in any year. For these traits we analyzed the effect of date and breeding system main effects on the mean per plant per day. For all other traits, date effects were omitted from the models.

We examined the effects of plant size, estimated as the number of flowers on a plant, on components of reproductive success, within and among populations. We also examined seed number as a covariate, testing its effect on all possible ovule fates and seed weight.

In 1984 and 1985, when each breeding system was represented by two populations, we tested for breeding system main effects using a nested model that estimated variation among populations within a single breeding system.

**Autogamy and pollen limitation**—To determine whether amount or quality of pollen limited reproduction, we imposed one of two pollination treatments on pairs of flowers on ten to 20 plants in each of two putative selfing and two putative outcrossing populations. The pollination treatments tested whether adding pollen increased seed set and whether the source of the additional pollen (self vs. other) affected seed set. If differences in reproductive success between selfing vs. more outcrossing populations were due to pollen limitation, we would predict a significant interaction between either addition of pollen and population breeding system or between source of pollen and population breeding system.

On one set of plants, where self pollen was applied, we excluded pollinators by placing cages of standard aluminum screening, approximately 8 inches in diameter and 6 inches in height, over each plant. One flower of each pair was allowed to set seed via spontaneous selfing. We hand-selfed the other flower using its own pollen. To ensure that no pollen was being introduced into the screen cages, we emasculated additional flowers in the bud stage on the caged plants.

On pairs of flowers on plants without cages, we examined whether natural levels of pollination might be limiting seed production. One flower of the pair was allowed to set seeds naturally. On the other flower we supplemented the natural pollen load by applying the pollen of two randomly chosen plants from within 1 m of the plant.

There was widespread infection of one of the selfing populations by a mustard rust (Albugo candida, Rollins, personal communication) in the year that these treatments were imposed; the rust killed many of the plants and lowered reproductive output of those that survived. We eliminated this population from the pollen limitation analysis, reducing the sample size for selfing populations.

**Resource limitation: distribution of fruits in seed set classes**—To determine whether resource limitation of reproduction was manifest as an overrepresentation of many-seeded vs. few-seeded fruits (Stephenson, 1981), we examined the distribution of fruits with different numbers of seeds. Since ovule number is variable in L. crassa, we examined seed set distribution in fruits with four ovules (53% of the 1,541 fruits collected in this study contained four ovules). A multidimensional G2 test (Feinberg, 1970; Meagher, personal communication) was used to determine if such fruits were randomly distributed into classes with one, two, three, and four seeds; we tested for distributional differences among the 3 years and between the two population breeding systems. We also examined the mean seed weight for each seed number class in four-ovuled fruits in both years for both breeding systems.

**Nonrandom maternal effects: ovule position effects**—We used a multidimensional G2 analysis to test the influence of ovule position within the ovary on fertilization, abortion, and seed weight. The fruits of Leavenworthia crassa are typical of cruciferous siliques in having two locules separated by a transparent septum. The position of each ovule or seed was determined in reference to other ovules or seeds within the same locule of the fruit, and only one locule per fruit was used in any analysis.
Table 1. Summary of nested analysis of variance of floral and reproductive traits in Leavenworthia crassa. Three to five individuals per family for each of 16–20 families from each of two populations were measured

<table>
<thead>
<tr>
<th>Trait</th>
<th>$N$ (plants)</th>
<th>Putative selfer (81)</th>
<th>Putative outcrosser (85)</th>
<th>Population effect P&lt;; % Variance</th>
<th>Family within population effect P&lt;; % Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent fruit*</td>
<td>108</td>
<td>0.48</td>
<td>0.25</td>
<td>0.001 (50.2%)</td>
<td>0.006 (17.7%)</td>
</tr>
<tr>
<td>Flower width (mm)</td>
<td>119</td>
<td>16.2</td>
<td>24.3</td>
<td>0.001 (91.4%)</td>
<td>0.006 (2.3%)</td>
</tr>
<tr>
<td>Petal length (mm)</td>
<td>119</td>
<td>12.2</td>
<td>16.9</td>
<td>0.001 (88.1%)</td>
<td>0.002 (4.4%)</td>
</tr>
<tr>
<td>Anther position</td>
<td>119</td>
<td>1.34</td>
<td>2.52</td>
<td>0.001 (56.4%)</td>
<td>0.001 (8.3%)</td>
</tr>
<tr>
<td>Days to flower</td>
<td>149</td>
<td>81.2</td>
<td>88.5</td>
<td>0.001 (28.0%)</td>
<td>0.003 (10.0%)</td>
</tr>
<tr>
<td>Total flowers</td>
<td>108</td>
<td>155.5</td>
<td>181.8</td>
<td>0.006 (9.4%)</td>
<td>0.179 (20.4%)</td>
</tr>
<tr>
<td>Mean seed number</td>
<td>59</td>
<td>2.74</td>
<td>1.86</td>
<td>0.001 (50.6%)</td>
<td>0.007 (22.9%)</td>
</tr>
<tr>
<td>Mean ovule number</td>
<td>56</td>
<td>5.05</td>
<td>5.07</td>
<td>0.582 (0.0%)</td>
<td>0.009 (39.4%)</td>
</tr>
<tr>
<td>Mean seed weight</td>
<td>79</td>
<td>1.84</td>
<td>1.74</td>
<td>0.052 (1.0%)</td>
<td>0.001 (46.0%)</td>
</tr>
</tbody>
</table>

* Arcsine square root transformed percent flowers that matured fruit.

RESULTS

Variation in plant mating system traits—In the Phytotron, plants from the putative outcrossing population had more and larger flowers, more extroverse anthers, set a lower proportion of fruits, set fewer seeds per fruit, and flowered later than did plants from a selfing population (Table 1). Ovule number did not significantly differ between populations with different breeding systems, and there were only marginally significant differences in mean seed weight per fruit, with plants from selfing populations tending to produce heavier seeds.

The family effect within populations was also significant for most traits, suggesting genetic variation within the populations for these traits. The family effect accounted for only a small proportion of the total among and within population variance components for flower and petal size, but a considerably larger proportion of total variance for such traits as flowering time, flower number, fruit and seed set, ovule number, and mean seed weight per fruit. For all traits, the variance among family means was greater in the outcrossing population than in the selfing population, with heterogeneity between population variances significant only for flower width ($P<0.01$, $F$-max test, Sokal and Rohlf, 1981).

Plants from an additional putative selfing population (#2) and outcrossing population (#4) showed no significant differences from plants in Populations 1 and 5, respectively, for three floral traits. Population 2 had small, monomorphically yellow flowers (mean width 16.7 mm, mean petal length 12.0 mm, and mean anther position 1.19). Population 4 was polymorphic for flower color, with mean flower width of 23.7 mm, mean petal length of 16.4 mm, and mean anther position of 2.4.

Over the 20-year period between our work and that of Lloyd, petal length appears virtually unchanged for the populations. Lloyd reported a mean petal length of 12.1 mm for race c5; we found that Population 5, one of Lloyd’s race c5 populations, had a mean petal length of 12.03 mm in 1984. Race c13, to which Population 2 belongs, had a petal length of 9.34 mm 20 years ago and a mean length of 9.12 mm in 1984. Lloyd found two anther position morphs in race c5, equivalent to our positions 2 and 3, while we measured a mean anther position of 2.54. For race c13, Lloyd reported only one anther position, our position 1, while we found an average anther position of 1.34. (Because Lloyd reported no variances, we cannot perform statistical analyses on these measures.)

The distribution of selfing did not map onto the interpopulational variation in moisture regime as simply as Solbrig and Rollins suggest. Our survey indicated that while outcrossing populations tended to have the greatest recorded soil depth, variation in soil depth in any one population was large and overlapped greatly with all other populations (ANOVA population breeding system $F = 63.73$, $P<0.0002$; population within breeding system $F = 62.54$, $P<0.0001$). The mean soil depth of one outcrossing population (#4, mean = 48.5 mm) was significantly less than that of the other outcrossing population (#7, mean = 92.4 mm, $P<0.0001$) and was not significantly different from that of one of the selfing populations (#2, mean = 38.6, $P<0.080$). Soil depth was negatively correlated with damage and mortality from freezing (Lyons, personal observation) and positively correlated with soil moisture content (Pearson product moment correlation = 0.167, $P<0.022$). As with soil depth, however, for soil moisture there was significant variation between populations of the same breeding system (ANOVA $F=25.21$, $P<0.0001$), as well as variation between selfing and outcrossing.
TABLE 2. Gene frequencies for electrophoretic markers and estimates of outcrossing rates for a putative selfing (Population 1) and a more outcrossing population (Population 5). See text for enzyme abbreviations. N = 17 families, 296 progeny in Population 1. N = 25 families, 403 progeny in Population 5. A minimum of four fruits were sampled from each mother (maximum = 13).

<table>
<thead>
<tr>
<th>Population 1</th>
<th>Ovule gene frequencies</th>
<th>Pollen gene frequencies</th>
<th>Number of progeny scored</th>
<th>Population outcrossing rate</th>
<th>Variance of estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>Allele</td>
<td>Allele</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>EST-1</td>
<td>0.912</td>
<td>0.088</td>
<td>0.884</td>
<td>0.116</td>
<td>296</td>
</tr>
<tr>
<td>GOT</td>
<td>0.706</td>
<td>0.294</td>
<td>0.771</td>
<td>0.229</td>
<td>251</td>
</tr>
<tr>
<td>ACP</td>
<td>0.912</td>
<td>0.088</td>
<td>0.681</td>
<td>0.319</td>
<td>82</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Population 5</th>
<th>Ovule gene frequencies</th>
<th>Pollen gene frequencies</th>
<th>Number of progeny scored</th>
<th>Population outcrossing rate</th>
<th>Variance of estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>Allele</td>
<td>Allele</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>EST-1</td>
<td>0.880</td>
<td>0.060</td>
<td>0.896</td>
<td>0.025</td>
<td>0.079</td>
</tr>
<tr>
<td>EST-2</td>
<td>0.680</td>
<td>0.240</td>
<td>0.556</td>
<td>0.314</td>
<td>0.130</td>
</tr>
<tr>
<td>PGI</td>
<td>0.800</td>
<td>0.200</td>
<td>0.797</td>
<td>0.164</td>
<td>0.040</td>
</tr>
<tr>
<td>PGM</td>
<td>0.860</td>
<td>0.140</td>
<td>0.815</td>
<td>0.185</td>
<td>0.224</td>
</tr>
<tr>
<td>ACP</td>
<td>0.860</td>
<td>0.140</td>
<td>0.852</td>
<td>0.148</td>
<td>0.281</td>
</tr>
</tbody>
</table>

populations (ANOVA $F = 14.40, P < 0.0001$). Selfing populations were at most 12 m higher in elevation than were outcrossing populations.

**Outcrossing estimates**—Population 1, the putative selfing population, had a significantly lower outcrossing rate ($t = 0.03$) than did the putatively outcrossing Population 5 ($t = 0.33$; $P < 0.001$ by $t$-test assuming unequal variances; Sokal and Rohlf, 1981) (Table 2). When only those variable enzymes common to both populations (EST and ACP) were used to estimate outcrossing, the putatively outcrossing population still had a significantly higher outcrossing rate ($t = 0.27$). The estimates presented in Table 2 are based on families with more than eight offspring; the estimates change very little (less than 10%) and remain significantly different when smaller families of four to seven progeny are included. There was also a lower level of electrophoretic variation in the selfing population; the mean number of heterozygous loci per individual per locus was 0.067 for the selfing population and 0.293 for the outcrossing population. (Other measures of electrophoretic variation, such as percent polymorphic loci and allelic diversity, are inappropriate because we surveyed only variable enzymes.)

For a number of the loci, the pollen and ovule gene frequencies vary considerably, suggesting that genotypes may contribute differentially to the gene pool as males vs. females.

In the selfing populations, individual plants ranged in outcrossing rate from 0.0 to 0.23; outcrossing events were detected for only four of 17 families, leading to the lower variance around the population outcrossing estimate (Fig. 2). In the more outcrossing population, individual plants ranged in outcrossing rate from 0 to 0.66; outcrossing events were detected for all but three of 25 families. The estimated outcrossing rates for the four plants in the outcrossing population with yellow flowers (mean $t = 0.41$, range = 0.0–0.66) were not significantly different from the outcrossing estimates for plants with white flowers (Kruskal-Wallis rank order test, Sokal and Rohlf, 1981).

Within populations, one-way regressions of maternal outcrossing rate on six offspring traits (petal length, flower width, anther position, flowering time, total number of flowers, and fruit set in the absence of pollinators) revealed only one correlation that approached significance ($P < 0.084$). This was the correlation between high maternal outcrossing rate and lower fruit set in the absence of pollinators, significant only in the outcrossing population.

Multiple regression of outcrossing rate onto the suite of six characters failed to explain a significant proportion of the variation in the selfing population ($P < 0.988$). In the outcrossing population, however, the multiple regression model approached significance ($P < 0.067$), with fruit set in the absence of pollinators, petal size, and flower width showing significant associations with maternal outcrossing rate with all six traits included in the model. Furthermore, the associations between outcrossing rate and floral character states found within the outcrossing population paralleled associations across populations; plants and populations with
Fig. 2. Frequency distribution of maternal of family outcrossing rate for a selfing population (POP = 1) and for a more outcrossing population (POP = 5).
lower fruit set in the absence of pollinators, larger petals, and larger flowers had higher outcrossing rates.

**Reproduction at the field sites—Patterns of natural reproduction**—In general we found that plants in more outcrossing populations tended to achieve less of their reproductive potential than did their counterparts in selfing populations. Over the 3-year period of this study, plants in outcrossing populations had a significantly larger proportion of unfertilized ovules and a significantly larger proportion of aborted ovules than did plants in selfing populations (Fig. 3; Table 3). However, the proportion of ovules matured into seeds did not differ between the two types of populations over the entire 3-year period, although in 1984, plants in outcrossing populations did mature a significantly smaller proportion of ovules than did plants in selfing populations (Fig. 3). This seeming contradiction occurred because plants from the selfing and outcrossing populations matured and aborted seeds differently in different years, yielding significant population breeding system by year interactions. Such year-to-year variation in reproductive patterns was marked; for any reproductive measure, significant differences detected over the entire 3-year period were never significant in all of the single years.

Over all 3 years, plants in selfing populations had significantly more flowers than did plants in outcrossing populations ($P < 0.015$, selfer mean = 8.2 flowers per plant; outcrosser mean = 6.2 flowers per plant). However, the difference in flower number between selfing and outcrossing populations also varied widely from year to year and was not significant for either 1984 or 1985 alone.

Plants in selfing populations also tended to have more ovules per ovary, although this effect was not significant when the covariate plant size, estimated as number of flowers, was included in the analysis. Plant size as a covariate had significant effects only on the number of ovules per fruit. Therefore, for all other traits, plant size effects were omitted from the models.

With lower seed abortion, higher fertilization, and a larger number of ovules per ovary, plants in selfing populations tended to mature a higher total number of seeds per fruit than did plants in outcrossing populations ($P < 0.06$; selfer mean = 3.56 seeds per fruit; outcrosser mean = 3.27 seeds per fruit). However, this effect was also temporarily variable, however, with significant date within year effects and year by population breeding system interaction effects on seed number matured per fruit.

In both 1985 and 1986, the 2 years for which we analyzed fruit set, plants in selfing populations tended to set a significantly larger proportion of fruits than did plants in outcrossing populations (Table 4). This effect was only significant in 1986. (No population by breeding
system effect was included in the model for 1985 because we excluded all plants from rust-infected Population 1 from the analysis and because there were no significant differences in fruit set between the two outcrossing populations.) Fruit set was also significantly affected by date of flowering in both years, with a decline in probability of fruit set occurring late in the flowering period (Table 4). In 1985, the significant population breeding system by date interaction reflected a more severe decline in fruit set over the flowering season in the outcrossing populations.

Seed number per fruit had a significant effect only on mean individual seed weight per fruit and so was included in the analysis of only that trait. Mean individual seed weight per fruit was significantly higher in the outcrossing populations both in 1985 and in 1986, when the effects of both date of flowering and seed number per fruit were used as covariates in the model (Table 5). In both selfing and outcrossing populations, seed weight decreased significantly with date in 1986.

We found little variation in reproductive pattern among populations grouped within either the selfing or outcrossing breeding system. Population 1 tended to have smaller plants than Population 2, though this was significant only in 1985. Population 4 tended to have smaller plants than Population 7, though this effect only approached significance in 1985 at

Table 3. Analysis of variance of proportion of ovules unfertilized, proportion of fertilized ovules aborted, and proportion of ovules matured into seeds

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Type IV SS</th>
<th>F</th>
<th>df</th>
<th>Type IV SS</th>
<th>F</th>
<th>df</th>
<th>Type IV SS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>All 3 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>1</td>
<td>0.07</td>
<td>3.68*</td>
<td>1</td>
<td>0.29</td>
<td>7.20**</td>
<td>1</td>
<td>0.06</td>
<td>1.43</td>
</tr>
<tr>
<td>Pop(PBS)</td>
<td>2</td>
<td>0.003</td>
<td>0.09</td>
<td>2</td>
<td>0.16</td>
<td>2.00</td>
<td>2</td>
<td>0.19</td>
<td>2.06</td>
</tr>
<tr>
<td>Year</td>
<td>2</td>
<td>0.07</td>
<td>1.92</td>
<td>2</td>
<td>0.10</td>
<td>2.39</td>
<td>2</td>
<td>0.17</td>
<td>1.88</td>
</tr>
<tr>
<td>PBS-year</td>
<td>2</td>
<td>0.07</td>
<td>1.78</td>
<td>2</td>
<td>0.23</td>
<td>5.82*</td>
<td>2</td>
<td>0.28</td>
<td>3.05*</td>
</tr>
<tr>
<td>Error</td>
<td>200</td>
<td>3.79</td>
<td></td>
<td>200</td>
<td>7.91</td>
<td></td>
<td>200</td>
<td>9.01</td>
<td></td>
</tr>
<tr>
<td>1984</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PBS</td>
<td>1</td>
<td>0.03</td>
<td>1.86</td>
<td>1</td>
<td>0.36</td>
<td>12.83**</td>
<td>1</td>
<td>0.56</td>
<td>16.40***</td>
</tr>
<tr>
<td>Pop(PBS)</td>
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<td>0.04</td>
<td>1.24</td>
<td>2</td>
<td>0.12</td>
<td>2.19</td>
<td>2</td>
<td>0.26</td>
<td>3.81*</td>
</tr>
<tr>
<td>Error</td>
<td>86</td>
<td>1.34</td>
<td></td>
<td>86</td>
<td>2.43</td>
<td></td>
<td>86</td>
<td>2.94</td>
<td></td>
</tr>
<tr>
<td>1985</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PBS</td>
<td>1</td>
<td>0.18</td>
<td>10.08**</td>
<td>1</td>
<td>0.08</td>
<td>1.61</td>
<td>1</td>
<td>0.01</td>
<td>0.10</td>
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<td>Pop(PBS)</td>
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<td>0.47</td>
<td>2</td>
<td>0.45</td>
<td>4.48*</td>
<td>2</td>
<td>0.35</td>
<td>2.37</td>
</tr>
<tr>
<td>Error</td>
<td>70</td>
<td>1.24</td>
<td></td>
<td>70</td>
<td>3.54</td>
<td></td>
<td>70</td>
<td>3.73</td>
<td></td>
</tr>
<tr>
<td>1986</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>1</td>
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<td>0.03</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
<td>1</td>
<td>0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>Error</td>
<td>42</td>
<td>1.16</td>
<td></td>
<td>42</td>
<td>1.65</td>
<td></td>
<td>42</td>
<td>1.91</td>
<td></td>
</tr>
</tbody>
</table>

* PBS: population breeding system.
* Pop(PBS): population within population breeding system.
* P < 0.05, ** P < 0.01, *** P < 0.001.

Table 4. Analysis of variance of proportion of flowers setting fruit. 1985 selfing breeding system does not include plants from rust-infected Population 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Source</th>
<th>df</th>
<th>Type III SS</th>
<th>F</th>
<th>Means by PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Selling</td>
</tr>
<tr>
<td>1985</td>
<td>PBS</td>
<td>1</td>
<td>0.25</td>
<td>1.78</td>
<td>85.2%</td>
</tr>
<tr>
<td></td>
<td>Date</td>
<td>1</td>
<td>3.34</td>
<td>24.30***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PBS-date</td>
<td>1</td>
<td>0.59</td>
<td>4.32*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>175</td>
<td>24.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1986</td>
<td>PBS</td>
<td>1</td>
<td>0.77</td>
<td>5.98*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Date</td>
<td>1</td>
<td>7.42</td>
<td>57.93***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PBS-date</td>
<td>1</td>
<td>0.34</td>
<td>2.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>187</td>
<td>23.98</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* PBS: population breeding system.
* P < 0.05, ** P < 0.01, *** P < 0.001.
the $P < 0.08$ level. For only one other trait in 1 year (aborted seeds in 1985) was there a significant difference between populations within a breeding system.

In Population 7, one of the outcrossing populations polymorphic for flower color, the plants with the less common yellow flower color matured a higher proportion of ovules into seeds ($F = 7.12$, df = 1, $N = 31$ plants, $P < 0.013$). The yellow-flowered plants tended to have lower proportions of unfertilized ovules and aborted ovules, although neither of these effects alone were significant. Flowers of the two colors were equally likely to set fruit, and seed weights were not significantly different.

In summary, plants in selfing populations were fertilized at a higher rate, had less seed abortion, tended to mature more seeds per fruit, had smaller seeds, had greater fruit set, and tended to have more flowers per plant than did plants in outcrossing populations.

Autogamy and pollen limitation—Only two of 96 emasculated flowers set seed, indicating that introduction of pollen into the screen cages was rare and that emasculation in the bud stage effectively removed pollen from the flower.

The addition of any type of pollen resulted in a significant increase in the proportion of fertilized ovules across the plants tested (Table 6). Although the means in Table 6 suggest that plants in outcrossing populations showed a greater increase after the addition of pollen than did plants in selfing populations, ANOVA indicated that the interaction between addition of pollen and population breeding system was not significant. This may have resulted because intrapopulational variation in response to added pollen is very large, as indicated by the highly significant plant within population effect. Thus, plants in both selfing and more outcrossing populations did not respond in different ways to the addition of pollen.

The source of added pollen (self vs. other) did not explain a significant amount of variation in ovule fertilization; nor did plants in selfing and more-outcrossing populations differ in response to added self or other pollen, that is, the source by population breeding system effect was not significant in Table 6B.

Resource limitation and distribution of fruits among seed set classes—Four-ovuled fruits were not randomly distributed among seed set classes; there were far more fruits with more seeds than there were fruits with few seeds ($G^2 = 532.6$, df = 18, $P < 0.0001$) in both population breeding systems and in all 3 years. There were significant breeding system by seed set distribution interactions ($G^2 = 35.3$, df = 9, $P < 0.0001$), with selfing populations showing a more pronounced skew in the abundance of many seeded fruits. There were also significant differences among the 3 years ($G^2 = 33.0$, df = 12, $P < 0.0001$), with 1986, the drought year, showing the least pronounced effects.
Table 6.  Means and ANOVA of proportion of ovules fertilized under two flower treatments

<table>
<thead>
<tr>
<th>A. Means by population breeding system (PBS)</th>
<th>Naturally selfed vs. natural plus supplemental pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneously selfed vs. hand-selfed</td>
<td>Selling</td>
</tr>
<tr>
<td></td>
<td>SPONT</td>
</tr>
<tr>
<td>0.908</td>
<td>0.899</td>
</tr>
<tr>
<td>n = 16</td>
<td>n = 16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. ANOVA on proportion of ovules fertilized (arcsine transformed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
</tr>
<tr>
<td>Plant(PBS)</td>
</tr>
<tr>
<td>PBS</td>
</tr>
<tr>
<td>Addition*</td>
</tr>
<tr>
<td>Source(Addition)*</td>
</tr>
<tr>
<td>Addition+PBS</td>
</tr>
<tr>
<td>Source(Addition)+PBS</td>
</tr>
<tr>
<td>Error</td>
</tr>
</tbody>
</table>

*SPO: spontaneously selfed.  
HAND: hand-selfed.  
NAT: naturally pollinated.  
SUPP: supplemental outcrossed pollen added.  
*Addition: (HAND plus SUPP) vs. (SPONT plus NAT).  
Source: (SPONT plus HAND) vs. (NAT plus SUPP).  
**P < 0.05, ***P < 0.001.

There were no significant population breeding system by year or population breeding system by year by seed number interactions.

In 1985, in selfing populations, four-ovuled fruits with more seeds tended to have larger seeds. In outcrossing populations, on the other hand, the largest mean seed weights occurred for fruits with only two seeds. In 1986 a similar pattern emerged, with many-seeded fruits having the heaviest seeds in selfing populations and few-seeded fruits having heavier seeds in the outcrossing populations.

Nonrandom maternal effects: ovule position effects—There were no significant differences in position effects between populations within selfing or outcrossing breeding system, so results from the two selfing populations were pooled, as were those from the two outcrossing populations. In fruits with three ovules per locule, one position, the middle ovule, is most or equally likely to be fertilized, least likely to abort, and most likely to be the heaviest seed (Table 7). In fruits with two ovules per locule, however, the top ovule is most likely to be fertilized, most likely to be the heavier seed, and most likely to abort. Population breeding system by position interactions are statistically significant in both populations for position of the heaviest seed, and in different populations for position of aborted and unfertilized ovules. However, in all cases the G² values for position effects are larger; that is, the position effects are more pronounced, in outcrossing populations than in selfing populations.

**DISCUSSION**

Variation in reproductive traits—A number of studies in natural populations have shown that there is genetic variation for floral traits that are likely to be correlated with outcrossing rates (e.g., flower color: Harding, 1970; Ennos and Clegg, 1983; flower size: Wyatt, 1984; degree of protandry: Schoen, 1982; percent cleistogamy: Clay, 1982; ray and disc floret morphology: Marshall and Abbott, 1984). Our findings indicate that floral and reproductive traits are also genetically based in *Leavenworthia crassa*. When plants were grown under conditions that minimized environmental variation, there was both significant between-population variation and also significant variation among families within populations for flower width, petal length, anther position, number of days to first flowering, and ability to set seed in the absence of pollinators. The fact that moderate levels of variation occur within a highly selfing population is concordant with both the theoretical predictions that variation in polygenic traits can be maintained under selfing (Lande and Schemske, 1985) and with the numerous empirical findings of genetic variation for traits in selfing species (summarized in Allard, Jain, and Workman, 1968). Our findings confirm and extend previous
demonstrations of wide variation in floral traits in *Leavenworthia crassa* populations (Rollins, 1963; Lloyd, 1964, 1965, 1969; Solbrig, 1972; Solbrig and Rollins, 1977). The floral variation still exists, it has a strong genetic component within as well as among populations, and means measured 20 years apart are virtually unchanged.

We did not find a strong correlation between patterns of population breeding system traits and patterns of population soil moisture or depth. Solbrig and Rollins (1977) suggested differences in soil moisture might have driven the evolution of selving in previously outcrossing lineages. They reasoned that in a dry habitat the plants that flowered earlier and matured seeds before the late spring dry spell would be favored. Outcrossing plants that flowered before the emergence time of native pollinators could not set seed whereas self-pollinating plants could. Morphological and physiological traits that allowed selving would therefore be favored in drier habitats. We estimated soil moisture only once. However, because we found wide variation in soil moisture and correlated soil depth both within single populations and among populations with the same breeding system attributes, we conclude that soil moisture is unlikely to have been the major force selecting for selving. Even if soil moisture were well correlated with breeding system traits, the correlation could also have resulted if selving plants had differentially colonized drier habitats.

**Outcrossing estimates** — Relatively few studies have shown how a genetically based trait influences genetic transmission (Rick, Holle, and Thorp, 1977; Schoen, 1982; Brown and Clegg, 1984; Schoen and Clegg, 1985). In *Leavenworthia crassa*, the population of plants with small, monomorphic flowers with no scent and introrse anthers had an approximately tenfold lower outcrossing rate than did the population of plants with larger, polymorphically colored flowers with strong scent and extrorse anthers.

Our results agree with those of Solbrig (1972) and Solbrig and Rollins (1977), who used protein electrophoresis to study breeding system attributes of several species in *Leavenworthia*. They found that for species and populations characterized by the same floral traits that we associated with outcrossing, there was a larger percentage of segregating families than for putatively selving species and populations. Segregation of progeny can result from either maternal heterozygosity (regardless of selving level) and/or from a higher maternal outcrossing rate. We have documented that in *L. crassa* populations with larger, polymorphically colored flowers and more extrorse anthers, there are higher levels of individual and population heterozygosity, as well as a higher outcrossing rate.

The intermediate level of outcrossing in Population 5 does not conform to the bimodal distribution of very high and very low outcrossing rates predicted by Lande and Schemske (1985; but see Waller, 1986; Uyenoyama and Antonovics, 1987; Holsinger, 1988). Further-
more, across the entire set of populations in the species, *Leavenworthia crassa* is likely to show an even wider range of outcrossing rates than do these two populations because our primary criteria for choosing populations was to study populations with enough self-compatibility (as assayed by Lloyd, 1964) to allow the comparison of selfed, outcrossed, and natural breeding systems (see Lyons, 1986).

There is also variance within populations in the outcrossing rates of different females. This variance is low in the selfing population, but much higher in the outcrossing population. Although within-population variation may be caused in part by environmental heterogeneity or by differences in local mate availability, we would predict that plants with large flowers and more extrorse anthers would be more outcrossed than neighboring plants with smaller flowers and more introrse anthers. Our indirect test of this prediction yielded a marginally significant regression of the suite of six traits on maternal outcrossing rate in the outcrossing population. We consider this result encouraging given that the regression could have been weakened either by the imperfect heritability of floral traits and/or by variation introduced by pollinator behavior. In models where a single gene affects outcrossing rate, stable intermediate levels of outcrossing are often associated with genetic polymorphism for the breeding system trait; a more direct assessment of how quantitative traits contributed to breeding system polymorphism and correlated outcrossing rates is warranted.

**Reproduction at the field sites—Patterns of natural reproduction**—Over the 3-year period of this study, plants in selfing populations had generally higher reproductive success. While a number of studies have compared reproductive success after controlled selfing and outcrossing (Schemske, 1983; Schoen, 1983; Karzon, 1989), fewer studies have documented patterns of reproductive success under natural selfing vs. outcrossing breeding system in closely related populations or taxa. In his survey of selfing vs. outcrossing annuals, Wiens (1984) found that inbreds tended to have a higher percentage of ovules developing into seeds than did outbreeders (*P* < 0.07). In a comparison of three lupine species, he also found that the selfing species had a higher percentage of fruits developing per flower and a higher number of seeds per fruit, both of which contributed to a higher relative reproductive success.

The pattern of higher reproductive success in selfing vs. outcrossing populations also matches theoretical expectations. In long-selfing populations, most deleterious recessives have been purged, so there is little inbreeding depression (Lande and Schemske, 1985). In selfing populations the developing seeds are also much more closely related to each other and to their mother than is the case in outcrossing populations (Westoby and Rice, 1982). Therefore, selection may be more likely to act on the more variable developing seeds in the outcrossing populations, yielding fewer seeds per ovule (see below).

The higher reproductive success in the selfing populations resulted from greater success at several different stages of reproduction. Selection acting on a single reproductive stage or trait is not likely responsible for the differences in reproductive success across breeding systems. Plants in outcrossing populations had one clear advantage over plants in selfing populations; they produced heavier seeds. The higher seed weight of outcrossed seeds suggests that while plants in selfing populations produce more seeds, the quality of offspring may differ between the two breeding systems. Finally, in no 1 year were more than half of these selfing advantages significant, illustrating the potential for episodic selection on reproductive traits (Uyenoyama and Antonovics, 1987).

In order to conclude that differences in reproductive success were due to variation in outcrossing rate and floral traits, we must demonstrate that the differences were not strictly environmental in nature. A reciprocal transplant, in which plants from both selfing and outcrossing populations are planted back into both populations, is the most straightforward way to distinguish environmental from breeding system effects. We conducted a reciprocal transplant experiment in 1986. Because nearly all of the 200 transplanted plants died in the severe spring drought, we must rely on other results to ascertain whether populational differences in reproductive success are attributable to breeding system or to some environmental difference in habitat.

One line of evidence that environmental variation and breeding system can unequally influence dimensions of reproductive success comes from a comparison of variation among populations within the same breeding system. While this source of variation was highly significant for both soil depth and soil moisture, there were rarely differences in reproductive trends between the two selfing populations or between the two outcrossing populations.

Comparing patterns of reproduction in field-grown plants with those in Phytotron-grown plants allowed us to further disentangle effects of breeding system from effects of environ-
mental variation. These comparisons contrast the two sets of plants in a common environment, although it is not the environment in which either set are normally found or in which either has evolved. Several of the reproductive variables assayed in the Phytotron (Table 1) showed the same patterns of variation as did field-grown plants (Tables 3–5). Under both conditions, selfing populations had a higher rate of fruit set and seed set, even though in the field there was likely to be more pollinator visitation in outcrossing populations than in selfing populations (Lloyd, 1965). Seed weight was higher in outcrossing populations, both in the Phytotron and in the field. Conversely, several traits showed different patterns or reversals between Phytotron and field. In the field, selfing plants had more ovules than did outcrossing plants, while in the Phytotron there was no significant difference. In the Phytotron, outcrossing plants had more flowers, while in the field, plants in selfing populations had more flowers.

These findings concur with those of Wiens (1984), who found that traits involving the number of reproductive units, such as flower number or ovule number, were more sensitive to environmental variation, while traits involving the fate of reproductive units, such as fruit set, seed set, and seed/ovule ratios, were less sensitive to environmental variation and were likely to be a function of the current and past breeding system. Because we know that floral traits have remained stable in these populations over several decades, and since we have found at least a marginally significant correlation between floral traits and outcrossing rate, we expect that outcrossing rates will also have remained relatively stable. We therefore interpret current reproductive patterns, as least for those traits involving the fate of reproductive units, such as fruit set and seed set, as an evolutionary result of population breeding system history.

_Pollen limitation_—Our results also provide data on the degree to which reproductive success in selfing vs. more-outcrossing populations is a function of three interacting factors: 1) pollen limitation (Schemske, 1977; McDade and Davidar, 1984); 2) resource limitation (Stephenson, 1981); and 3) the relative roles of male and female function (Lloyd, 1980; Bawa and Webb, 1984).

Ideally, one would study pollen limitation by quantifying the amount of pollen deposited on each stigma (Waser, 1983; McDade and Davidar, 1984; Lyons et al., 1989). In the absence of this type of detailed information, we quantified relative levels of fertilization when the amount and source of pollen was varied. While adding pollen did lead to increased fertilization, the increase was similar in outcrossing and selfing populations. Thus the amount of pollen delivered per flower does not appear to explain the differences in reproductive success between selfing and more-outcrossing populations.

By adding pollen to flowers we tested for pollen limitation on a per flower basis. In outcrossing species, if the amount of pollen delivered per flower is insufficient to fertilize all ovules in the flower, plants that produce more flowers may be favored (Bawa and Webb, 1984). If this had been the case, the outcrossing plants would have had a lower proportion of ovules fertilized (therefore appearing pollen limited on a per flower basis), but might have compensated by producing many more flowers to achieve numbers of fertilized ovules equal to the number for selfing plants. While plants in outcrossing populations did show a lower proportion of fertilized ovules, they also tended to have fewer flowers per plant and an overall lower number of matured seeds. Thus plants in outcrossing populations do not show a compensatory response to low levels of fertilization.

Because the source of pollen (self vs. other) neither influenced the level of fertilization nor differed in its effect on selfing vs. outcrossing populations, we concluded that lower fertilization in the outcrossing populations was not due to higher levels of self-incompatibility. Although Lloyd (1965, 1967, 1968) showed reduced seed set after selfing in some outcrossing populations of this species, other studies of fertilization after selfing vs. outcrossing did not reveal great differences in self-incompatability between these selfing and more-outcrossing populations (Lyons, 1986). Finally, the variation in fertilization among plants within a population was highly significant. While some of this variation may be attributable to within-population variation in self- or cross-compatibility, part of this variation is likely attributable to environmental variation within a population. Lyons (1986) conducted crosses on plants from these populations growing in the field and in the Phytotron and found that variation in fertilization was much higher in field-grown plants.

_Resource limitation_—Resources limit reproduction to some extent in these populations, as evidenced by decreases in fruit set and seed weight with date, an excess of multisepaled fruits, and heavier multisepaled fruits. How-
ever, there is conflicting evidence on whether decreased reproductive success in the outcrossing populations is due to more severe resource limitation there. While fruit set declines over the season more steeply for the outcrossing populations in 1 year, resource limitation appears to be more severe in the selfing populations where there is a greater skew in the distribution of fruits in multiseeded classes.

Nonrandom maternal effects: ovule position effects—All of our indices have measured reproductive success achieved through female function. Neither the pollen limitation hypothesis nor the resource limitation hypothesis can unequivocally account for differences in reproductive success across populations with different mating histories. This may be because both of these hypotheses equate the incomplete development of all potential female reproductive structures with some degree of reproductive failure.

It is possible that overproduction of flowers and fruits confers an advantage to a plant via 1) an increase in male function (but see Campbell, 1989), 2) the opportunity to take advantage of exceptionally good growing conditions ("bet-hedging"), and/or 3) the potential for nonrandom abortion of fruits or seeds of low quality (Bawa and Webb, 1984). This study did not address the first two possible advantages (although the second would seem advantageous for this species exposed to wide yearly climatological variation manifest during this study as severe freezing, droughts, and tornados).

Both the excess of fruits with high seed number and the significant within-fruit position effects indicate that processes acting in female reproduction are not random with respect to fruits within plants nor to ovules within fruits. The congruence of nonrandom patterns, where ovules in one position are most likely to be fertilized, least likely to be aborted, and most likely to develop into the heaviest seed, is particularly striking. Furthermore, the tendency for the position effects to be stronger in the outcrossing populations suggests that nonrandom processes may exert a larger force on reproduction there. Hossae and Valero (1988) found similar nonrandom position effects for fertilization, seed abortion, and fruit maturation in several Lathyrus species. They interpreted the pattern of embryo abortion as a balance between the advantages of an early start and higher genetic quality for seeds nearest the pollen source vs. the resource advantage (seen as lower abortion) for seeds closest to the source of maternal nutrition. They also found that the nonrandom position effects were greater in the more outcrossing species than in the selfing one. Whatever the mechanism yielding the pattern, both our results and those of Hossae and Valero support Casper's (1984) suggestion that the potential for selective embryo abortion would be more important in outcrossed than in inagamous species.

A number of authors have argued that such nonrandom patterns result when female choice and sexual selection operate (Bertin and Stephenson, 1983; Willson and Burley, 1983; Marshall and Ellstrand, 1986, 1988). Both female choice and sexual selection are easy to postulate and difficult to demonstrate in plants (Charleworth, Schemske, and Sork, 1987; Lyons et al., 1989).

It is not difficult to construct a scenario in which the nonrandom development of seeds in Leavenworthia crassa might reflect female choice, or even female choice of "good genes" that confer higher fitness on the progeny (Boake, 1986). The growth rate of pollen tubes is a function of the complementarity between genes acting in pollen, stigmatic, and stylar tissues and the fastest growing tubes fertilize the middle ovules. Due either to active female choice or to passive choice based on past selection for a permanent shunt of more resources to central ovules, the seeds fertilized by the best males are the largest and rarely abort. Thus, the advantage of large seed size is conferred upon the progeny that already embody the best complement of maternal and paternal genomes. Because the variation in potential mates is larger in outcrossing populations, there exist both greater opportunities for discrimination as well as greater potential benefits from discrimination among mates.

While this scenario is consistent with our results, we have insufficient evidence to confirm it and we hope to better understand the processes yielding the above described patterns. To determine whether sexual selection is at work and whether there is potential for evolutionary response to that selection in Leavenworthia crassa populations requires information on genotype-specific fertilization of ovules in different positions (Hill and Lord, 1986; Mazer, Snow, and Stanton, 1986), on the correlations between traits expressed by haploid pollen and resultant offspring (Mulecay and Mulecay, 1975), on the significance of seed weight for fitness (Stanton, 1984), and on the heritability of male traits that act during reproduction (Mazer, 1987). Equally important would be experimental studies on the nature of nonrandom transmission in sets of controlled crosses that vary the opportunity for
interaction between different female and male tissues (Lyons et al., 1989). Furthermore, very little is known for this species or for any other species regarding the anatomical constraints on pathways for resource partitioning, source-sink relations, and control of resources in developing fruits, and the nature of genotype-specific, rather than position-specific (Casper, 1984; Hossuert and Valero, 1988; Marshall and Ellstrand, 1988) seed abortion.

The unequal ovule and pollen gene frequencies indicate that some genotypes are contributing differentially to the gene pool via male and female function. This finding, plus the nonrandom patterns of seed and fruit development that differ between types of populations, clearly warrants further study to elucidate why plants in outcropping populations tend to have lower levels of female reproductive success than do plants in selving populations.

LITERATURE CITED


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