THE ROLE OF INBREEDING DEPRESSION IN MAINTAINING THE MIXED MATING SYSTEM OF THE COMMON MORNING GLORY, *IPOMOEA PURPUREA*

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Abstract.—Theoretical studies show that, although inbreeding depression (ID) will counterbalance the transmission advantage of selfing, it can only maintain a mixed mating system in plants when at least one of the following two conditions is met: (1) there is a positive association between selfing rates and the level of ID; and (2) ID is greater than 0.5 for the female component of fitness, while the average ID for male and female fitness is less than 0.3. This study tests whether these two conditions hold in the common morning glory, *Ipomoea purpurea*, which has a mixed mating system with 30% self-fertilization. Inbreeding depression was found in all but one fitness component measured in two groups of plants with distinct anther-stigma distances (ASD), a character that influences selfing rates. However, when examined separately, a negative association was found between selfing rates and ID; plants with large ASD (low-selfing-rate genotypes) tended to have higher ID than ones with small ASD (high-selfing-rate genotypes). Furthermore, the overall lifetime ID for male (12.5%) and female (24%) components of fitness, averaged across two ASD groups, were lower than what is necessary for ID to maintain an evolutionarily stable mixed mating system. Therefore, although inbreeding depression contributes to balancing the transmission advantage of selfing, it is not likely to be the primary mechanism maintaining the mixed mating system of *I. purpurea*. The contribution of other mechanisms is discussed.

**Key words.**—Herkogamy, inbreeding depression, *Ipomoea purpurea*, mating system.

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Understanding the evolution of mating systems has been of major interest to plant population biologists. One aspect of mating system evolution that has been subject to particularly intense examination is the evolution of selfing versus outcrossing. It has long been recognized that selfing can be favored under certain ecological conditions (e.g., colonizing new habitats; Baker 1955, 1967; Stebbin 1957; unreliable pollinators: Darwin 1876; Lloyd 1979; Holsinger 1996; and local adaptation of plant populations: Antonovics 1968). However, because these ecological advantages probably cannot explain the evolution of selfing in all, or even the majority of species, a more general advantage of selfing has been emphasized: An individual that selfs transmits two copies of mating-system alleles to its selfed progeny, whereas an individual that outcrosses transmits only one copy through ovules. Plants that self-fertilize thus gain an automatic transmission advantage (Fisher 1941; Jain 1976; Maynard Smith 1978). As a result of this advantage, if there are no countering forces, mutant alleles that enhance selfing are expected to invade an outcrossing population and eventually become fixed (see reviews in Charlesworth and Charlesworth 1987; Jarne and Charlesworth 1993; Uyenoyama et al. 1993). In light of this advantage, much interest has focused on the question of what prevents all plants from evolving complete selfing.

One often-proposed answer to this question is that in many plant species, inbreeding depression (ID) opposes the transmission advantage associated with selfing. Early theoretical investigations (Nagylaki 1976; Maynard Smith 1977; Lloyd 1979; Charlesworth 1980; Feldman and Christiansen 1984; Holsinger et al. 1984; Lande and Schemske 1985) suggested the existence of a threshold level of ID, equal to one-half (but see Charlesworth 1980; Holsinger 1988, 1991; Uyenoyama and Waller 1991 a,b,c). According to these models, the expected frequency distribution of selfing rates would be bimodal, with one mode corresponding to complete selfing when ID is smaller than 0.5 and the other to complete outcrossing when ID is greater than 0.5. Although many plant species seem to predominantly self and others predominantly outcross (Schemske and Lande 1985), these models do not explain the persistence of mixed mating systems, particularly in species in which there is genetic variation for selfing rates.

More recent models suggest two possible mechanisms by which ID may stabilize a mixed mating system. The first mechanism involves associations between genotypes at mating-system loci and genotypes at fitness (ID) loci within a population (Campbell 1986; Holsinger 1988; Charlesworth and Charlesworth 1990; Uyenoyama and Waller 1991b; Latta and Ritland 1993, 1994). For this mechanism to stabilize a polymorphism, associations must be such that the magnitude of ID is greater for genotypes that have higher selfing rates because it is this additional fitness loss to ID that counteracts the transmission advantage associated with these genotypes. Whether such associations are likely to form depends in theory on the genetic causes of ID (Schultz and Willis 1995; see reviews by Charlesworth and Charlesworth 1987 and Uyenoyama et al. 1993).

Because in no plant species have both genes that affect selling and genes that contribute to ID been characterized, it has not been possible to assess directly the nature of any associations that form between mating system and fitness loci in natural populations. An alternative approach has been to ask whether families with differences in floral characters thought to influence selfing rates differ in the magnitude of ID. Although several investigators have documented variation among families in the magnitude of ID (Nason and Ells...
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Trand 1995; Pray and Goodnight 1995; Holtsford 1996; Carr et al. 1997), only one study has attempted to determine whether this variation is genetically correlated with characters affecting selfing. Carr et al. (1997) used herkogamy level, the distance between anthers and stigma, and autogamy level, the ability to produce seeds without pollinators, to represent lineages with different levels of selfing, but failed to find associations between these characters and levels of ID. There is thus insufficient empirical information to determine the extent and nature of associations that form between mating system loci and loci affecting ID, and thus whether these associations contribute to stabilizing mixed mating systems.

The second mechanism by which ID may stabilize a selfing-rate polymorphism arises in cases in which ID is more severe for the female component of fitness than for the male component of fitness. Specifically, Rausher and Chang (1999) have shown, using a model in which ID effects are fixed, that a necessary condition for such stabilization is that ID for female fitness must be greater than 0.5, while the average ID for male and female fitness must be less than 0.5. To date, ID for male fitness components in hermaphroditic plants have either been neglected in studies or have only been measured indirectly in the greenhouse (Willis 1993; Damgaard and Loeschcke 1994; Carr and Dudash 1995, 1997; del Castillo 1998); therefore, it is unclear how frequently this mechanism may contribute to stabilization of a mixed mating system in natural populations.

The primary objective of the experiments described here was to ascertain whether either of these two mechanisms involving ID may contribute to stabilizing a mixed mating system in the common morning glory, *Ipomoea purpurea* Roth (Convolvulaceae). The outcrossing rate in natural populations of this species ranges between roughly 60% and 80% (Ennos 1981; Brown and Clegg 1984). Moreover, genetic variation in both flower color (Epperson and Clegg 1987; Rausher et al. 1993; Fry and Rausher 1997) and anther-stigma separation (herkogamy) (Chang and Rausher 1998) influence selfing rate in nature. In this investigation we concentrate on the effects of anther-stigma separation. Like many plant species in which there is variation in herkogamy (Holtsford and Ellstrand 1992; Motten and Antonovics 1992; Beilousoff and Shore 1995; Carr et al. 1997; Karron et al. 1997), *I. purpurea* genotypes with smaller anther-stigma distances have higher selfing rates than genotypes with greater separation (Chang and Rausher 1998; see also Ennos 1981). The transmission advantage associated with selfing is therefore expected to favor the evolution of smaller anther-stigma distances (hereafter ASDs). The observation that genotypes with large ASDs persist in natural populations (Chang and Rausher 1998), however, indicates that some selection pressure opposes fixation of the more-selfing genotypes. We ask here whether ID contributes to that opposing selection. Specifically, we ask three questions. Is the populationwide magnitude of ID greater than the theoretical threshold predicted by many models to be necessary for ID by itself to oppose the evolution of selfing? Do genotypes with small ASDs exhibit greater ID than genotypes with larger distances, as would be required if associations between mating-system loci and fitness loci contribute to stabilization of a mixed mating system, as manifested by an intermediate ASD? Are there differences in the magnitude of ID between male and female fitness components of the type that could stabilize the mating system?

**Materials and Methods**

**Study Organism**

*Ipomoea purpurea* is an annual, weedy species that grows in disturbed areas such as gardens, roadsides, and abandoned or poorly weeded agricultural fields. In North Carolina, seedlings usually emerge between early May and August, when the fields are first plowed and ground-cover vegetation is removed. Plants begin flowering about six weeks after germination and seeds mature in about four weeks. Individual flowers open early in the morning and normally wither by early to midafternoon of the same day. Bumblebees are the sole pollinators (Ennos 1981; Stucky 1985; Rausher et al. 1993). Plants flower until they are killed by the first hard frost in late fall. In natural populations, morning glories send out stems along the surface of the ground or twine up other vegetation, such as corn and soybean plants. Patch (or population) size varies a great deal, ranging from fewer than 10 plants to more than 1000 plants (pers. obs.). Colonizing seeds are believed to be transferred by harvesting machinery moving from field to field (Pear 1983).

**Breeding Design and Production of Experimental Seeds**

To determine the relationship between degree of anther-stigma separation and ID, we chose plants with extreme values of ASDs and used these plants to produce selfed and outcrossed experimental seed. Initially, seeds were collected from *I. purpurea* plants growing on a tobacco farm in Durham County, North Carolina, in 1993. Plants from which seeds were collected were sampled haphazardly from the plants in the population, ensuring that the distance between any two plants was at least five meters. These collected seeds were grown in a greenhouse, and the ASD of each plant was recorded as the distance between the tallest anther and the stigmatic surface. ASD was recorded as positive if the stigma was above the tallest anther. The mean ASD of these parental plants, calculated from at least five different flowers open on different days, was $-1.0 \pm 1.3$ mm (SD); in other words, the tallest anthers were on average about 1 mm above the stigmatic surface.

To obtain two classes of parental plants with extreme values of ASD, we chose the 18% of the plants (60 of 326 plants) with the greatest ASD and the 18% with the smallest ASD to form the parental generation for the experimental seed (Fig. 1). Plants in these two classes differed in average ASD by 4 mm, or about three standard deviations. Experimental seeds representing these two ASD classes were obtained by mating the parental plants within ASD classes. To permit assessment of the magnitude of ID on the male component of fitness, a presumed neutral allozyme marker, phosphoglucurono mutase (*Pgm*) was employed in these crosses to generate experimental seed of known genotype at this locus. Specifically, in all crosses, only plants that were homozygous for the slow *Pgm* allele (i.e., were genotype SS) were used as seed parents, whereas all outcrossed pollen parents were homozygous for the fast allele (FF). Each maternal plant was both self-fertilized...
Fig. 1. Frequency distribution of ASD in the parental generation. Positive values represent flowers with the anthers below the stigma (i.e., large ASD) and vice versa.

and outcrossed to at least three pollen donors to produce experimental seeds. Flowers for outcrossing were emasculated before opening to prevent unwanted selfing. This breeding design resulted in four classes of seeds: outcrossed and selfed seeds by small-ASD parents and outcrossed and selfed seeds by large-ASD parents. All of the selfed seeds were SS at Pgm, whereas all outcrossed seeds were FS. The experimental seeds were weighed individually to a precision of 0.1 mg before planting.

Field Experiment

A total of 1749 experimental seeds was scarified and planted in the field in early June 1995. Of these seeds, 877 were from 20 seed parents of the small-ASD group and 872 were from 20 seed parents of the large-ASD group. The numbers of seeds used differed slightly among seed parents due to differences in productivity; nevertheless, the ratio between selfed and outcrossed seeds was maintained at approximately 1.4:1. This ratio was chosen to maximize the number of seeds used.

The experimental plot was an old agricultural field in Durham County, North Carolina. Seeds were organized into three spatial blocks; within each block, experimental seeds were randomly assigned to different locations on a 1-m² grid system. Four fitness components of these experimental plants were measured: (1) proportion of seeds emerging; (2) survivorship of emerged seedlings to produce at least one seed; (3) number of flowers and seeds produced by surviving plants; and (4) mean pollen contribution of surviving selfed and outcrossed plants to the outcross pollen pool. Native vegetation was mowed twice during the experiment to control the cotton rat population in the experimental plot. In addition, all native I. purpurea were removed to prevent pollen contamination when estimating pollen contribution. Emergence of the experimental seeds was censused daily.

Lifetime seed production was measured for each of the reproducing plants. Individual fruits were collected separately and the identity of the maternal plants was recorded along with the date of collection. The field experiment ended when a hard frost killed all of the plants in early November, after which the number of seeds in each fruit and the total number of seeds produced by each experimental plant were counted. Because the size of a seed produced by a plant may affect progeny fitness, the mean seed size for each fruit was also estimated.

Several life-history traits were also recorded during the field experiment to examine the expression of ID in various life history characters. Seedling size was estimated by measuring the length of each leaf and then estimating total leaf area using an estimated relationship between leaf area and leaf length. The date of producing the first flower and the number of flowers produced each day were also recorded.

Ipomoea purpurea showed a strong response to artificial selection on ASD in the greenhouse (Chang and Rausher 1998). However, it was still desirable to determine whether this response was also manifested in the field to assure the assumed distinction between the herkogamy classes in this experiment. To do so, the ASDs of the experimental plants were sampled between 0800 h and 1000 h on five days during the course of experiment. A thin metal rod with a ruler having a 0.5-mm scale was placed straight into the flower to measure the ASD of each flower without disturbing pollen.

Pollen Contribution to the Outcross Pollen Pool

To estimate pollen contribution by selfed and outcrossed experimental plants to the outcross pollen pool, pollen-trapping trials were performed on 32 separate days during September and October. For each trial, several potted plants that were homozygous at the Pgm locus were used as pollen trapping plants. The number of trap-plants used in each trial depended on the availability of plants in the greenhouse. On average, about six trap-plants (ranging between four and nine plants) were used in each trial. Most of the trap-plants had between one and three flowers (mean ± SD = 2.3 ± 0.6) open during the trial, with rare exceptions that up to six flowers were open on a single plant. All of the flowers on the trap-plants were emasculated the evening before the trial, before flowers opened. The trap-plants were then evenly distributed among the experimental plants, and pollination was allowed to occur naturally after the flowers opened the next day. A trial ended when the flowers had withered, usually by early afternoon, at which time trap-plants were removed from the field and placed in a greenhouse, where fruits were allowed to develop. Each fruit was labeled with date of pollination and the maternal plant’s Pgm genotype. The mature seeds were scored for their genotypes at the Pgm locus. A total of 799 seeds were produced from these pollen-trapping trials and all of them were included in this analysis.

One mechanism that could explain any differential outcross success revealed by the pollen-trapping trials is differential pollen production. To evaluate the possibility that this mechanism might be operating, one anther per flower was collected on five days during the field experiment. Because there is evidence that the pollen production was not different among the five anthers within a flower (M. D. Rausher, unpubl. data), only the anther at the intermediate height was collected during the afternoon the day before flowers opened. Collected
anthers were left in 96-well cell-culture dishes until the next day. The few anthers that did not dehisce were excluded from the analysis. Pollen grains were transferred to glass slides using the basic fuchsins glycerin-jelly technique described in Kearns and Inouye (1993). The grains were preserved on the slides and their numbers were counted later using Image One software on an IBM personal computer.

Statistical Analyses

Assessment of Variation in Herkogamy.—To confirm that plants belonging to the different herkogamy classes in the greenhouse had distinctive ASDs in the field, an analysis of covariance was performed on the field-measured ASD. The independent variables included the herkogamy class; maternal parent (dams), which was nested within the herkogamy classes; the sampling dates as class variables; and the initial seed mass as a covariate. The effect of dam was treated as random, whereas herkogamy class and sampling date were considered to be fixed. Throughout this study, when performing significance tests for random effects, the appropriate mean squares used as the error terms were determined according to their expected values (Sokal and Rohlf 1981). This analysis was carried out using PROC GLM of the SAS statistical software package (SAS Institute 1990).

Effects of Inbreeding on Size of Experimental Seeds.—For each capsule produced by the parental plants in the greenhouse, an analysis of variance was performed on the total seed mass to determine whether the breeding type (selfed vs. outcrossed) affected fitness during seed development. Because some studies suggest that a trade-off may exist between seed number and seed mass (Smith and Fretwell 1974), we also performed similar analyses on the mean seed number per capsule and individual seed mass.

Fitness Components.—Emergence and survivorship were analyzed by maximum-likelihood analysis of covariance using PROC CATMOD of the SAS software package. Initial seed mass was used as a covariate in this and subsequent analyses.

The effect of inbreeding on both seed number and mean seed size produced by surviving plants was examined using analysis of covariance. Seed number was log-transformed to satisfy normality assumptions, whereas the mean size of the seeds produced by each experimental plant was square-root transformed. For both analyses, the factors evaluated included the number in the analysis for survivorship plus dam (nested within herkogamy class) and the interaction between breeding type and dam. To examine the expression of ID on life-history characters, a multivariate analysis of covariance was performed on the measured life-history characters. We also examined the effects of inbreeding on overall measures of lifetime male and female fitness, in which plants that reproduced were assigned a fitness equal to the number of flowers (for male fitness) or seeds (for female fitness) they produced, whereas seeds that were planted but did not reproduce were assigned a fitness equal to zero. Both male and female lifetime-fitness were transformed using the transformation ln(fitness + 1).

To determine whether ID resulted in differential male-outcross success, we evaluated the null hypothesis that experi-

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling date</td>
<td>9</td>
<td>132.3</td>
<td>6.66</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Herkogamy class</td>
<td>1</td>
<td>61.2</td>
<td>12.86</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dam(herk.)</td>
<td>39</td>
<td>185.7</td>
<td>2.16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Initial seed mass</td>
<td>1</td>
<td>2.51</td>
<td>1.14</td>
<td>0.29</td>
</tr>
<tr>
<td>Error</td>
<td>328</td>
<td>724.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>378</td>
<td>1108.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

mental plants from both breeding types had the same per flower probability of fertilizing ovules on the trap-plants. If this hypothesis is correct, one would expect to see that the F-allele frequency among the successful pollen grains that sired ovules of the trap-plants during a trapping trial (referred to as observed F allele frequency hereafter) to be the same as the F allele frequency among the flowers bloomed during that day (referred to as expected F allele frequency hereafter).

The expected allele frequency can be simply calculated using the following formula: \( O_i/(2O_i + 2S_i) \), where \( O_i \) and \( S_i \) are the number of flowers produced by outcrossed (Pgm genotype FS) and selfed (Pgm genotype SS) individuals on date \( i \), respectively. To estimate the observed allele frequency, the genotypes of both the trap-plants and the seeds they produced were compared. For each seed, the allele carried by the pollen grain that fertilized it was determined by subtracting the allele type contributed by the trap-plant (through ovules) from the seed genotype. For example, if the genotype of a seed was FS and the trap-plant was SS, then the pollen must have carried an F allele. To calculate the observed F allele frequency for an individual trial, we first calculated, for each trap-plant, the frequency of the F allele among the pollen that sired seeds on that trap-plant. The mean of the F allele frequencies for all of the trap-plants in that trial was then calculated to represent the overall observed F allele frequency for that trial. Because this method weighed every trap-plant in a trial equally, regardless of the number of seeds it produced, it avoided potential biases due to differential seed production among the trap-plants. We then compared the observed frequencies of the 32 trials with their corresponding expected frequencies using a paired t-test. A significant difference between the expected and observed frequencies would lead us to reject the null hypothesis.

To determine whether pollen production varied between the breeding types, an analysis of covariance was performed. Because the sample size was relatively small (189 flowers), only a subset of the independent variables was included in this analysis: sampling date, herkogamy classes, breeding type, and herkogamy × breeding type.

RESULTS

ASD in the Field

Selection of plants with extreme values of ASD was successful in creating two groups of experimental plants with divergent ASD in the field (Table 1, parental herkogamy effect). The offspring of small-ASD parents, in which the anther
TABLE 2. Analyses of variance for the effect of inbreeding on pregermination characters: seed number and total mass per maternal capsule and mean seed mass for experimental seeds. Breeding type: selfed versus outcrossed; other sources of variation as in Table 1. Significant effects are shown in bold.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>(a) Seed number/capsule</th>
<th></th>
<th></th>
<th></th>
<th>(b) Mean seed mass</th>
<th></th>
<th></th>
<th></th>
<th>(c) Total weight per capsule</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>Type III SS</td>
<td>F</td>
<td>P</td>
<td>df</td>
<td>Type III SS</td>
<td>F</td>
<td>P</td>
<td>df</td>
<td>Type III SS</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Breeding type</td>
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<td>0.11</td>
<td>0.05</td>
<td>0.82</td>
<td>1</td>
<td>0.003</td>
<td>0.13</td>
<td>0.72</td>
<td>1</td>
<td>0.001</td>
<td>0.01</td>
<td>0.94</td>
</tr>
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<td>Herkogamy class</td>
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<td>0.17</td>
<td>0.06</td>
<td>0.80</td>
<td>1</td>
<td>0.18</td>
<td>0.65</td>
<td>0.43</td>
<td>1</td>
<td>0.33</td>
<td>1.55</td>
<td>0.21</td>
</tr>
<tr>
<td>Dam(herk.)</td>
<td>39</td>
<td>109.18</td>
<td>1.16</td>
<td>0.32</td>
<td>39</td>
<td>11.38</td>
<td>12.86</td>
<td>&lt;0.001</td>
<td>39</td>
<td>23.13</td>
<td>2.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Breeding × herkogamy</td>
<td>1</td>
<td>2.69</td>
<td>1.12</td>
<td>0.29</td>
<td>1</td>
<td>0.07</td>
<td>3.09</td>
<td>0.08</td>
<td>1</td>
<td>0.36</td>
<td>1.71</td>
<td>0.19</td>
</tr>
<tr>
<td>Breeding × dam(herk.)</td>
<td>39</td>
<td>93.89</td>
<td>1.33</td>
<td>0.09</td>
<td>39</td>
<td>0.89</td>
<td>0.48</td>
<td>0.99</td>
<td>39</td>
<td>7.56</td>
<td>0.92</td>
<td>0.62</td>
</tr>
<tr>
<td>Error</td>
<td>519</td>
<td>937.48</td>
<td></td>
<td></td>
<td>519</td>
<td>24.33</td>
<td></td>
<td></td>
<td>519</td>
<td>109.83</td>
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<tr>
<td>Corrected total</td>
<td>600</td>
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<td></td>
<td></td>
<td>600</td>
<td>38.73</td>
<td></td>
<td></td>
<td>600</td>
<td>144.58</td>
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</table>

partially overlapped the stigma, exhibited an average ASD in the field of 0.5 ± 0.12 mm (mean ± SE). In contrast, the average ASD was 1.5 ± 0.12 mm for the large-ASD group. This response to selection indicates that the two groups of selected parents, as well as the offspring they produced, differed genetically for ASD. Plants produced by different seed parents (dams) within a parental herkogamy class also exhibited significantly different ASDs, suggesting the presence of genetic variation for ASD within the selected herkogamy classes. These results were consistent with the previous reports of high heritability for ASD as measured in greenhouse-grown plants (Ennos 1981; Chang and Rausher 1998).

Effects of Inbreeding on Size of Experimental Seeds

Inbreeding depression may be expressed either during seed development (pregermination) on the maternal plant, affecting seed characters such as seed size or number per capsule, or postgermination, affecting standard fitness components such as survival and seed production. We first examined whether ID occurred during maturation of the experimental seed in the greenhouse. Among the treatments evaluated, the seed parent (dam) from which the experimental seeds were produced was the only factor with a significant effect on the total seed mass produced per capsule (Table 2c). This difference seemed to be caused by the size of the individual seeds rather than the number of seeds in each capsule (Table 2a, b), indicating the possibility of a maternal effect on seed size. Seed size was therefore used as a covariate when analyzing the fitness for the experimental seeds. However, breeding type did not have any effect on either seed number or the average seed mass per capsule (Table 2); consequently, there is no evidence of ID operating during seed development.

Fitness Components

Emergence

Of the 1749 experimental seeds planted in the field, only 988 (56%) emerged. For emergence, there was a significant Breeding type × herkogamy interaction (Table 3a): Emergence was equal for selfed and outcrossed seeds for large-ASD plants, whereas for small-ASD plants percent emergence was 11% lower in outcrossed seeds.

Female Fitness

Survival to Produce Seeds and Seed Production.—A total of 756 seedlings survived to produce at least one seed by the end of the field experiment (76% postemergence survival to seed production). Although inbreeding did not reduce emergence probability, it reduced postemergence survival (Table 3b). Specifically, survival of selfed seeds was approximately 10% lower than that of outcrossed seeds for both large- and small-ASD plants.

Among plants that produced seeds, there was a marked effect of inbreeding on seed production (Table 4b). Outcrossed individuals from both large- and small-ASD groups produced similar amounts of seeds (open bars in Fig. 2a), providing no evidence for seed discounting. Selfed plants from large- and small-ASD groups exhibited 22.7% and 9.4% reductions in their seed productions compared to outcrossed plants of the same herkogamy group (Fig. 2a). Despite that the magnitude of this ID appeared to be larger in large-ASD

TABLE 3. Maximum-likelihood analyses of variance for probability of planted seeds emerging (emergence) and for survival of emerged seedlings to produce flowers or seeds. Sources of variation as in Tables 1 and 2. Significant effects are shown in bold.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>(a) Emergence</th>
<th></th>
<th></th>
<th></th>
<th>(b) Survival to produce seeds</th>
<th></th>
<th></th>
<th></th>
<th>(c) Survival to produce flowers</th>
<th></th>
<th></th>
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<tbody>
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<td></td>
<td>df</td>
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<td>P</td>
<td>df</td>
<td>Chi-square</td>
<td>P</td>
<td>df</td>
<td>Chi-square</td>
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<tr>
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<td>0.24</td>
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<td>0.01</td>
<td>0.93</td>
<td>1</td>
<td>10.89</td>
<td>0.01</td>
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<td>2</td>
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<td>0.61</td>
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<td>1</td>
<td>0.61</td>
<td>0.43</td>
<td>1</td>
<td>0.13</td>
<td>0.72</td>
<td>1</td>
<td>3.49</td>
<td>0.06</td>
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<tr>
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<td>0.59</td>
<td>1</td>
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<td>0.74</td>
<td>1</td>
<td>3.49</td>
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<tr>
<td>Breeding × herkogamy</td>
<td>1</td>
<td>4.35</td>
<td>0.037</td>
<td>1</td>
<td>14.97</td>
<td>&lt;0.001</td>
<td>826</td>
<td>936.34</td>
<td>0.004</td>
<td>826</td>
<td>630.07</td>
<td>1.00</td>
</tr>
<tr>
<td>Initial seed mass</td>
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<td>0.002</td>
<td>0.90</td>
<td>826</td>
<td>936.34</td>
<td>0.004</td>
<td>826</td>
<td>630.07</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Likelihood ratio</td>
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<td>1771.8</td>
<td>0.001</td>
<td>826</td>
<td>936.34</td>
<td>0.004</td>
<td>826</td>
<td>630.07</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Analyses of covariance for (a) the mean seed mass produced by the surviving plants; (b) the number of seeds produced by surviving plants; and (c) the total lifetime female fitness including individuals that did not emerge or survive. The mean seed mass was square-root transformed; the seed numbers were ln transformed, and the lifetime female fitness was ln(n + 1) transformed. Sources of variation as in Tables 1 and 2. Significant effects are shown in bold.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>(a) Mean seed mass</th>
<th>(b) Seed number produced</th>
<th>(c) Total lifetime female fitness</th>
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</thead>
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<tr>
<td></td>
<td>df</td>
<td>SS</td>
<td>F</td>
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<tr>
<td>Block</td>
<td>2</td>
<td>4.42</td>
<td>6.61</td>
</tr>
<tr>
<td>Breeding type</td>
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<td>1.28</td>
</tr>
<tr>
<td>Herkogamy class</td>
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<td>0.28</td>
<td>1.17</td>
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<td>Dam(herk.)</td>
<td>39</td>
<td>14.85</td>
<td>1.72</td>
</tr>
<tr>
<td>Breeding × herkogamy</td>
<td>1</td>
<td>0.24</td>
<td>1.09</td>
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<tr>
<td>Breeding × dam(herk.)</td>
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<td>8.64</td>
<td>0.86</td>
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<tr>
<td>Initial seed mass</td>
<td>1</td>
<td>0.79</td>
<td>3.08</td>
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<tr>
<td>Error</td>
<td>671</td>
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<td>671</td>
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<tr>
<td>Corrected total</td>
<td>755</td>
<td>202.49</td>
<td>755</td>
</tr>
</tbody>
</table>

(a) Seed production

(b) Flower production

![Graphs showing seed and flower production](image)

Fig. 2. Inbreeding depression in male and female components of fitness for plants in the large- and small-ASD groups. The values graphed for seed (a) and flower production (b) are the least-square means and their standard error estimated from the ANCOVAs (Tables 4, 5) with nontransformed data. Dark bars, selfed seeds; white bars, outcrossed seeds. The numbers associated with each ASD group in the graph are the relative performance of selfed and outcrossed seeds:

$$\text{relative performance} = \frac{W_o - W_s}{W_s} \times 100\%,$$

where $W_o$ and $W_s$ are the number of seeds (or flowers) produced by outcrossed and selfed plants and $W_s$ is the larger of the two values in the numerator (Agren and Schemske 1993). plants than in small-ASD plants, this trend was not significant (Table 4b, breeding × herkogamy effect). Neither breeding type, herkogamy class, nor their interaction had any detectable effect on the mean size of an individual seed (Table 4a).

**Composite Female Fitness.**—The composite estimate of lifetime female fitness, which included plants producing no seeds, exhibited a similar pattern. Across both herkogamy classes, inbred plants produced an average of 24% fewer seeds than outcrossed plants (Table 4b, Fig. 3a). Not surprisingly, this difference is very similar to the sum of the effects of inbreeding on postemergence survival (10%) and on seed production by plants producing at least one seed (16%). The magnitude of ID appeared to be somewhat greater for large-ASD plants than for small-ASD plants. Although this trend was not statistically significant, the $F$-value associated with this effect was relatively high (2.15), indicating that a difference in magnitude of ID between the two herkogamy classes cannot be ruled out with much certainty.

**Male Fitness**

**Survival to Produce Flowers.**—Most of the emerged seedlings survived to produce at least one flower (91% postgermination survival to flower production). Although inbreeding reduced the probability that emerged seedlings survived to flower production (Table 3c), the overall reduction of seedling survivorship due to inbreeding was minimal (3.4%).

**Pollen Contribution to Outcrossed Pollen Pool and Flower Production.**—Results from the pollen-trapping trials provided little evidence to indicate that selfed and outcrossed plants differed in their success at transmitting pollen to other plants, when success is measured on a per flower basis. The observed frequency of the F allele in the progeny of the trap-plants was on average only 0.02 less than the frequency expected assuming individual flowers on selfed and outcrossed plants had an equal probability of siring those progeny, a difference that was not statistically significant ($t = 0.63, P > 0.05, n = 32$). Anthers from selfed and outcrossed plants also produced similar numbers of pollen grains (Table 5a). Consequently, the male outcross success of selfed and outcrossed plants would be equal if plants of the two herkogamy classes produced the same number of flowers. However, for the plants that reproduced, lifetime flower production was significantly affected by inbreeding (Table 5b, Fig. 2b). Averaged across
Composite Male Fitness.—We also calculated an estimate of relative lifetime male fitness, as reflected by flower number, which includes zeros for plants that did not flower. The overall magnitude of ID, averaged across herkogamy classes, was 12.5%, approximately half the value for lifetime female fitness. Although ANCOVA did not reveal ID for lifetime male fitness to be significantly different from zero (breeding type effect, Table 3c), we suspect that this result arises from reduced power to detect significance because of a bimodal distribution of residuals. Given the significant ID for flower number when only plants that flowered were analyzed, we believe that the point estimate of ID for lifetime male fitness is probably real. As with lifetime female fitness, most of this ID for lifetime male fitness occurred in large-ASD plants, with a minimal amount of ID occurring in small-ASD plants (Fig. 3b).

**DISCUSSION**

**Inbreeding Depression and the Evolution of Anther-Stigma Distance**

As generally recognized, selfing itself confers a transmission advantage on genotypes with high selfing rates, which provides a component of selection that favors an increase in the frequencies of those genotypes (Fisher 1941; Nagylaki 1976; Maynard Smith 1977; Charlesworth 1980; Holsinger et al. 1984; Lande and Schemske 1985). Any explanation for evolutionarily stable variation in selfing rates must therefore account for two phenomena: (1) the absence of countervailing selective forces of sufficient magnitude to overcome the transmission advantage when genotypes with high selfing rates are at low frequencies; and (2) the presence of such forces when genotypes with high selfing rates are at high frequencies. The results presented here, along with previous work (Chang and Rausher 1998), clarify the contribution of ID to understanding how these two phenomena act to maintain intermediate levels of ASD in *L. purpurea*.

Genotypes with small ASD exhibit increased rates of selfing (Chang and Rausher 1998). Moreover, when these genotypes are rare, small-ASD genotypes actually exhibit slightly greater per flower male outcross success than large-ASD genotypes (Chang and Rausher 1998). Consequently, pollen discounting does not appear to contribute to preventing an increase in the frequencies of small-ASD genotypes when

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**Table 5.** Analyses of covariance for (a) pollen grains produced; (b) total flower production by surviving plants; and (c) total lifetime male fitness, including plants that did not emerge or survive to produce flowers. The number of flowers produced was ln transformed and the lifetime male fitness was ln(n + 1) transformed. Sources of variation as in Tables 1 and 2. Significant effects are shown in bold.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>(a) Pollen grains produced</th>
<th>(b) Flower production</th>
<th>(c) Total lifetime male fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>Type III SS</td>
<td>F</td>
</tr>
<tr>
<td>Sampling date</td>
<td>4</td>
<td>14,554.56</td>
<td>4.02</td>
</tr>
<tr>
<td>Breeding type</td>
<td>1</td>
<td>937.31</td>
<td>1.04</td>
</tr>
<tr>
<td>Herkogamy class</td>
<td>1</td>
<td>125.85</td>
<td>0.14</td>
</tr>
<tr>
<td>Dam(herk.)</td>
<td>39</td>
<td>18.78</td>
<td>1.42</td>
</tr>
<tr>
<td>Breeding × herkogamy</td>
<td>1</td>
<td>549.65</td>
<td>0.61</td>
</tr>
<tr>
<td>Breeding × dam(herk.)</td>
<td>39</td>
<td>14.11</td>
<td>1.02</td>
</tr>
<tr>
<td>Initial seed mass</td>
<td>1</td>
<td>1194.94</td>
<td>1.32</td>
</tr>
<tr>
<td>Error</td>
<td>180</td>
<td>162,981.23</td>
<td>814</td>
</tr>
<tr>
<td>Corrected total</td>
<td>188</td>
<td>179,409.81</td>
<td>898</td>
</tr>
</tbody>
</table>
rare. Theoretical analyses of the evolution of selfing indicate that under these conditions, ID must normally be smaller than 50% in order for the transmission advantage of selfing to provide a net increase in the frequency of small-ASD genotypes (Maynard Smith 1977; Lloyd 1979; Charlesworth 1980; Feldman and Christiansen 1984; Holsinger et al. 1984). This condition appears to be satisfied in *Ipomoea purpurea*. Our estimates of ID for the lifetime male and female components of fitness, which include effects of ID on viability as well as reproductive success, are both under 25%. Their average, which is the relevant measure of ID when the two components differ (Rauscher and Chang 1999), is thus less than half the threshold value considered necessary to completely counteract the transmission advantage of selfing. It thus appears that, although ID occurs in *Ipomoea purpurea*, it is not of sufficient magnitude by itself to prevent the initial spread of small-ASD genotypes.

When small-ASD genotypes are common and large-ASD genotypes rare, the large-ASD genotypes exhibit greater male outcross success than small-ASD genotypes; the magnitude of this apparent pollen discounting appears to be sufficient to prevent fixation of small-ASD genotypes (Chang and Rauscher 1998). ID thus does not need to be invoked to explain protection of the large-ASD genotypes. However, in our previous experiments, pollen discounting did not operate when the frequency of small-ASD genotypes was below about 0.95, whereas in natural populations these genotypes occur at much lower frequencies. It thus appears that some selective pressure in addition to pollen discounting must be operating to maintain a higher frequency of large-ASD genotypes than expected from a balance between discounting and the transmission advantage of increased selfing. Although our lack of understanding of the genetic basis for ASD prevents quantitative modeling, it seems likely that the substantial ID occurring in *Ipomoea purpurea* contributes to shifting the equilibrium frequencies to lower levels (Johnston 1998). The magnitude of ID in this species thus helps to explain both why small-ASD genotypes increase when rare and why they do not increase to near fixation.

As described in the introduction, several models of the evolution of selfing that explicitly allow evolution at loci responsible for ID indicate that ID may contribute to stabilization of a polymorphism for selfing rate if associations form between genotypes at selfing-rate loci and genotypes at inbreeding-depression loci (Holsinger 1988; Charlesworth and Charlesworth 1990; Uyenoyama and Waller 1991b; Latta and Ritland 1993, 1994). The nature of any associations that develop will depend on the exact genetic basis of ID, but alleles conferring increased selfing may become associated with alleles at ID loci conferring reduced fitness. Such an association could stabilize a selfing rate polymorphism because the greater fitness of the genotypes with lower selfing rates counteracts the transmission advantage associated with increased selfing. Empirically, this identity disequilibrium would be detected as differences in the magnitude of ID in lineages that differ in selfing rate. Specifically for the case of *Ipomoea purpurea*, it would be detected as a greater ID in small-ASD plants than in large-ASD plants. However, our experiment revealed a consistent, although not significant, trend in the opposite direction, indicating that it is unlikely that identity associations between alleles at selfing-rate loci and alleles at ID loci contribute to stabilizing the mixed mating system of *Ipomoea purpurea*.

In contrast with previous models, Schultz and Willis (1995) found that associations between the selfing history and ID due to mutation-selection balance would be weak, difficult to detect within a single population, and usually not strong enough to prevent fixation of an allele conferring increased selfing. Their results could thus be taken to suggest that it is unrealistic a priori to expect such associations to ever stabilize a mixed mating system, as well as to indicate why we failed to detect any statistically significant associations. However, their model considers only ID produced by deleterious recessive or partially recessive mutations, but not that produced by overdominance. Moreover, although Schultz and Willis reported no cases in which a mixed mating system was evolutionarily stable, their simulations do not rule out the possibility of such cases. Their model is thus silent on the magnitude of associations that could develop if a mixed mating system were stabilized by such associations. Given that previous models indicate that such associations may be strong, the question of whether they stabilize a mixed mating system in any particular population is an empirical issue. In this context, the evidence from our experiment seems to rule out the contribution of such associations to maintaining intermediate levels of ASD in *Ipomoea purpurea*.

Two caveats regarding this conclusion are necessary. First, this conclusion assumes that when the mating system is controlled by more than one locus, the expected genotypic associations will be the same as predicted in extant models. Most extant models have examined the associations between only one mating-system locus and one or more fitness loci (but see Latta and Ritland 1993). ASD is likely to be a quantitative character; therefore, the expected association between this character and the level of ID may be complicated by its polygenic control. Second, it should be recognized that the (nonsignificant) associations between herkogamy and magnitude of ID constitute only indirect evidence for the existence of identity disequilibrium between loci controlling selfing rate and fitness loci. Obtaining more direct evidence would require similar experiments in which selfing and fitness loci were examined directly.

Our results also indicate that a second mechanism by which ID may favor intermediate selfing rates probably does not operate in *Ipomoea purpurea*. Unlike traditional models that assume ID affects viability (e.g., Maynard Smith 1977; Lande and Schemske 1985; Charlesworth and Charlesworth 1990; Charlesworth et al. 1990; Uyenoyama and Waller 1991 a,b,c), Rauscher and Chang (1999) have shown that when the magnitude of ID differs for the lifetime male and female components of fitness, certain combination of ID for male and female fitness components allow evolutionarily stable mixed mating systems. In particular, a necessary condition for this stabilization is that ID is greater than 50% for female fitness, but the average ID for male and female fitness is less than 50%. This possibility seems to be ruled out for ASD in *Ipomoea purpurea*, however, because the magnitude of ID for female fitness is far less than 50%. One caveat associated with this conclusion is that our estimates of ID may be lower than the true value because the seeds used in the field experiment
were produced in the greenhouse. They may have thus enjoyed a relatively benign maternal environment during development, which could have mitigated any ID expressed during that stage (Wolfe 1993). However, it is difficult for us to envision, however, the maternal environment in the greenhouse being so much more benign that it would mitigate ID at this stage by 20–25%, the magnitude of additional ID that would be required to increase total female ID to more than 50%.

**Male versus Female Inbreeding Depression**

Recent analyses have indicated that in selfing species, effects of ID tend to be more severe in the later stages of the life cycle, particularly during growth and reproduction; whereas in outcrossing species, effects of ID are found in most of the life-history stages (Hubbard and Schemske 1996). Our results appear to lie between these two trends. Some degree of ID was found in each of the life-history stages, with higher degrees expressed in later stages. In addition, our results suggest that the magnitude of ID for lifetime female fitness was approximately twice as great as for lifetime male fitness. These observations indicate that most models of the influence of ID on the evolution of selfing incorporate an assumption that may be inappropriate: ID operates on viability, and thus equally on males and females. Presently, the consequences of making this assumption are theoretically unclear.

The assessment of the effects of selfing on male fitness has rarely been attempted. As long as the magnitude of ID is similar for male and female fitness components (e.g., Damgaard and Loeschcke 1994; Carr and Dudash 1995), this deficiency should not be problematic. ID for female fitness then accurately reflects the average ID for the two sexual components of fitness, which determines whether selfing or outcrossing is favored (Rausher and Chang 1999). However, when ID differs substantially for male and female fitness, failure to estimate ID for male fitness can lead to invalid inferences about the effects of ID on the evolution of selfing. For example, consider a situation in which ID is 60% for female fitness, but only 30% for male fitness. Taking female fitness as representative of overall ID would lead to the conclusion that ID is strong enough to prevent the evolution of selfing (ignoring identity disequilibrium). In contrast, the true average ID for male and female fitness is 45%, which is not sufficient to prevent the evolution of selfing (Rausher and Chang 1999).

Although the difference in ID between male and female fitness components in *I. purpurea* is not great, there is no theoretical reason to believe differences could not be large in other species. Such a difference requires that there be some loci affecting only male fitness and other loci affecting only female fitness. It also requires either that there are more ID loci contributing to one of the two fitness components or that the contribution of an average locus differs between ID loci affecting male fitness and ID loci affecting female fitness. Unfortunately, too little is known about the genetics of reproductive characters in plants to determine whether either of these requirements is frequently met. Nevertheless, a few investigations have revealed that male and female fitness traits are to some extent controlled by different sets of genes (Coen and Meyerowitz 1991; Meagher 1992; Yanofsky 1995) and that there are genes only expressed in pollen and not in the sporophytes (e.g., Willig et al. 1988; Mascarenhas 1989; Ottaviano and Mulcahy 1989). Moreover, several studies have detected substantial differences in the magnitude of ID for components of male (pollen number, pollen viability) and female (ovule number, seed number) fitness (Robertson et al. 1994; Carr and Dudash 1995, 1997; del Castillo 1998). Although these studies, along with ours, suggest that between-gender differences in the magnitude of ID may occur somewhat frequently, their general importance for the evolution of mating systems have yet to be explored. In particular, we believe it would be valuable for investigators to examine the relative magnitude of male and female ID in species with mixed mating systems. When doing so, care must be taken to estimate the effects of ID on total male fitness, not just on components such as pollen production or pollen viability, because effects of inbreeding on pollen transmission could substantially influence total male fitness. The approach here provides one method for obtaining such estimates.

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