

ANALYSIS OF INBREEDING DEPRESSION IN MIXED-MATING PLANTS PROVIDES EVIDENCE FOR SELECTIVE INTERFERENCE AND STABLE MIXED MATING

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Hermaphroditic individuals can produce both selfed and outcrossed progeny, termed mixed mating. General theory predicts that mixed-mating populations should evolve quickly toward high rates of selfing, driven by rapid purging of genetic load and loss of inbreeding depression (ID), but the substantial number of mixed-mating species observed in nature calls this prediction into question. Lower average ID reported for selfing than for outcrossing populations is consistent with purging and suggests that mixed-mating taxa in evolutionary transition will have intermediate ID. We compared the magnitude of ID from published estimates for highly selfing ($r > 0.8$), mixed-mating ($0.2 \leq r \leq 0.8$), and highly outcrossing ($r < 0.2$) plant populations across 58 species. We found that mixed-mating and outcrossing taxa have equally high average lifetime ID ($\delta = 0.58$ and 0.54 , respectively) and similar ID at each of four life-cycle stages. These results are not consistent with evolution toward selfing in most mixed-mating taxa. We suggest that prevention of purging by selective interference could explain stable mixed mating in many natural populations. We identify critical gaps in the empirical data on ID and outline key approaches to filling them.

KEY WORDS: Age-specific expression of inbreeding depression, gynodioecy, mating-system evolution, outcrossing, purging, selfing.

The most general theory for mating-system evolution in hermaphrodites suggests that populations will evolve to complete selfing or complete outcrossing, depending on the balance between automatic selection favoring self-fertilization (Fisher 1941) and inbreeding depression (ID) favoring outcrossing (Lloyd 1979; Lande and Schemske 1985), assuming that increased selfing does not result in a disproportionate decrease in outcrossing (Holsinger et al. 1984). The theory also predicts that evolutionary transition from outcrossing to selfing should occur quickly (within hundreds of generations) because of the rapid purging of genetic load and the loss of ID when an outcrossing population begins to self (Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Charlesworth et al. 1990). One hypothesis to explain the occurrence of mixed-mating populations, in which individuals produce both self and outcrossed progeny, is that they are in evolutionary transition from outcrossing to selfing. If purging is fast, the evolutionary transition from outcrossing to selfing should occur quickly, and mixed-mating populations should be rare. Despite these expectations, a recent tally of mating-system estimates for 345 plants species (Goodwillie et al. 2005) revealed that 42% exhibited mixed mating, defined as selfing rates between 20% and 80%. Although sampling bias against both selfing and highly outcrossing species may inflate the frequency of mixed-mating populations (Igic and Kohn 2006; Goodwillie et al. 2010), the number of species reported to show mixed mating is not trivial (Barrett and Eckert 1990; Goodwillie et al. 2005; Jarne and Auld 2006), and calls into question the status of mixed-mating populations as transitional, and therefore the sufficiency of the theory to explain the observed distribution of mating systems in hermaphroditic species.

The expression of genetic load upon inbreeding, termed ID, has been a central focus of both theory and empirical studies of mating-system evolution for decades (e.g., see Lloyd 1979; Lande and Schemske 1985; Charlesworth and Charlesworth 1987; but see Holsinger 1991). In a review of empirical estimates of ID, Husband and Schemske (1996) found a negative relationship between ID and primary selfing rate (r) and that primarily outcrossing populations ($r < 0.45$) as a group had significantly stronger ID than primarily selfing populations ($r > 0.55$). These results are consistent with decline in ID with the evolution of greater selfing, and clearly predict that if mixed-mating populations are evolving toward greater selfing, they should express lower ID than outcrossing populations. However, here, we present analyses of an updated database of ID and mating system that demonstrate that the average ID of mixed-mating taxa is not intermediate but rather is as large as that for outcrossing taxa. This result indicates a nontrivial frequency of mixed mating that is evolutionarily stable.

EXPECTATIONS FOR ID IN MIXED-MATING TAXA

The wide range of variation in selfing rates among closely related species and even populations of the same species indicates that mating system is evolutionarily labile. Observations of mixed mating might therefore be explained as stages in the evolutionary transition between the extremes of complete selfing and complete outcrossing (Schemske and Lande 1985; Weber and Goodwillie 2009; Ness et al. 2010). If most mixed mating can be explained as evolutionary transitions, then the general theory is sufficient to explain much of the observed distribution of mating systems of natural populations. Alternatively, mixed mating might be evolutionarily stable for a substantial number of species or populations. Numerous models have proposed conditions that could explain evolutionary stability of mixed mating (reviewed by Goodwillie et al. 2005), but determining which of the conditions they invoke are relevant to natural populations remains an empirical question that has not been satisfactorily resolved for even a single species.

To determine definitively whether mixed mating is evolutionarily stable in a given population requires measuring the relationship between the selfing rates of individuals and their lifetime fitness. Stabilizing selection favoring intermediate selfing would indicate stable mixed mating, whereas directional selection would suggest that the mating system is in evolutionary transition toward greater selfing or greater outcrossing. Measuring the fitness function for the selfing rate of individuals within a population is technically challenging at best because it requires estimating fitness gained through pollen and seed. No direct estimates of this relationship are currently available, but ID is one of the key components of the fitness function, and its magnitude has been estimated for many natural populations.

ID, the lower fitness of self than of outcross progeny, is driven primarily by recurrent, largely recessive deleterious mutations that arise in all populations (Charlesworth and Charlesworth 1987; Johnston and Schoen 1995; Charlesworth and Willis 2009). Self-fertilization exposes these mutations to selection and thus reduces the magnitude of ID (Charlesworth and Charlesworth 1987; Husband and Schemske 1996; Ritland 1996; Byers and Waller 1999). Consequently, the expression of ID is expected to be lower in more highly selfing populations and to accelerate directional selection toward greater selfing. In contrast, genetic load is expected to accumulate in outcrossing populations and to favor the evolution of inbreeding avoidance. Empirical data demonstrating significantly greater ID in primarily outcrossing than in primarily selfing taxa of plants (Husband and Schemske 1996) and of hermaphroditic snails (Escobar et al. 2011) support these expectations.

Based on genetic theory for the dynamics of ID and the empirical evidence supporting purging, we would expect that if most mixed-mating taxa were evolving toward a more extreme mating

system, the mean ID expressed by these taxa would lie between the average ID of primarily outcrossing taxa and primarily selfing taxa. Strongly deleterious or partially dominant alleles are purged rapidly under most conditions when an outcrossing population begins to self-fertilize (Lande and Schemske 1985; Charlesworth and Charlesworth 1987, 1995; Charlesworth 2003; simulation results below), suggesting that ID should also decline rapidly. Taxa evolving from selfing toward outcrossing may be accumulating load but are unlikely to have accumulated as much as stable outcrossing species, on average, because even modest amounts of selfing will purge some new mutations. Although the mating system of any single taxon could be either stable or evolving, if most mixed-mating taxa are evolving toward a more extreme mating system, then we expect the average ID of mixed-mating taxa to be less than that of primarily outcrossing and greater than that of primarily selfing taxa.

Because evolutionary transition toward greater selfing is thought to occur more frequently than that toward outcrossing (Stebbins 1957; Takebayashi and Morrell 2001; Igic et al. 2006), the majority of mixed-mating taxa should be in transition toward greater selfing. Assuming that purging occurs, the mean ID for mixed-mating species in transition should be closer to the mean for selfers than to the mean for outcrossers because more taxa will have purged than have accumulated new mutations. In addition, the particularly low average ID between fertilization and seed germination expressed by primarily selfing relative to outcrossing taxa (Husband and Schemske 1996) suggests that ID expressed between fertilization and dispersal is purged most quickly. Such early life-cycle ID in mixed-mating species that are in evolutionary transition may therefore be as low as that of primarily selfing species.

Similarity in average ID for mixed-mating and selfing species is not sufficient to indicate that most mixed-mating taxa are in evolutionary transition, because conditions, such as pollen discounting (e.g., see, Holsinger 1991; Johnston 1998) and temporally fluctuating ID (Cheptou and Mathias 2001, but see Porcher et al. 2009), could maintain mixed mating at low to moderate ID. In contrast, similar mean ID for mixed-mating and outcrossing taxa would suggest a mechanism that inhibits purging and therefore limits a major source of selection toward greater selfing.

Of the mechanisms proposed to contribute to stable mixed mating, only one includes conditions under which ID as great as that of primarily outcrossing taxa could be maintained in a population despite moderate to high self-fertilization (Lande et al. 1994). When the effects of deleterious mutations at multiple loci combine to prevent any selfed offspring from surviving to reproduce, there is no opportunity for selection against homozygous deleterious mutations. Consequently, even recessive lethals will not be purged. Lande et al. (1994) dubbed this phenomenon “selective interference” and Porcher and Lande (2005a) demonstrated that

it can generate a threshold selfing rate below which purging is completely ineffective. If self-pollen arrives at receptive stigmas and self-fertilization is not prevented by self-incompatibility, then taxa affected by selective interference can have stable intermediate rates of selfing at fertilization and express strong ID. Because it prevents purging, selective interference is expected to maintain ID in partially selfing taxa that is as great as that of purely outcrossing taxa,

Simulation of purging

We conducted numerical simulation to confirm that, if ID is purged after initiation of selfing, this process occurs rapidly. We simulated the process of purging using the inbreeding history approximation of Kondrashov’s model for evolution of the genetic load (Kondrashov 1985; Kelly 2007). The Kondrashov model has been used extensively to explore the relationship between equilibrium ID and selfing rate (e.g., by Charlesworth et al. 1990; Porcher and Lande 2005b; Kelly 2007; Johnston et al. 2009). Deleterious mutations occur at an effectively infinite number of loci at a genomic rate U in an infinite population. The dominance coefficient (h) and strength of selection (s) are the same for all loci. ID for a specified selfing rate (r) is determined by the distribution of numbers of heterozygous and homozygous mutations per individual. We used a simulation program (written by John K. Kelly) that allows specification of the genomic mutation rate, dominance coefficient for mutations, and strength of selection against mutations. We determine the ID in each generation after a transition from complete outcrossing to the new frequency of selfing ($r' = 0.99, 0.95$, and 0.80). We simulated the change in ID expected when an outcrossing population begins to self for a range of parameters reflecting estimates of mutation rate, selection against mutations, and dominance coefficients. Because we assume a fixed frequency of selfing, any value of r for which $0 < r < 1$ indicates mixed mating. The broader definitions of outcrossing and selfing that we apply to our empirical data reflect the expected effects of measurement error and the environment (e.g., pollinator availability and behavior) on estimates of selfing.

Our simulations indicate that, if purging happens, most of it occurs rapidly (Fig. 1, Appendix A). Purging can be completely prevented by strong selection ($s = 1$) or nearly complete recessivity ($h = 0.02$), as has been shown previously (Lande et al., 1994; Kelly 2007; Fig. 1A). For parameters describing moderately and mildly deleterious mutations, purging occurred and was completed within a few hundred to a few thousand generations (Fig. 1B,C). Purging was faster when the magnitude of selection or dominance was greater, when the new selfing rate was higher, and when the rate of mutation was lower (Appendix A). Our results for a range of estimates suggest that conclusions regarding purging are robust to variation in the values of parameters describing mutations (Appendix A). The rapid purging indicated by our

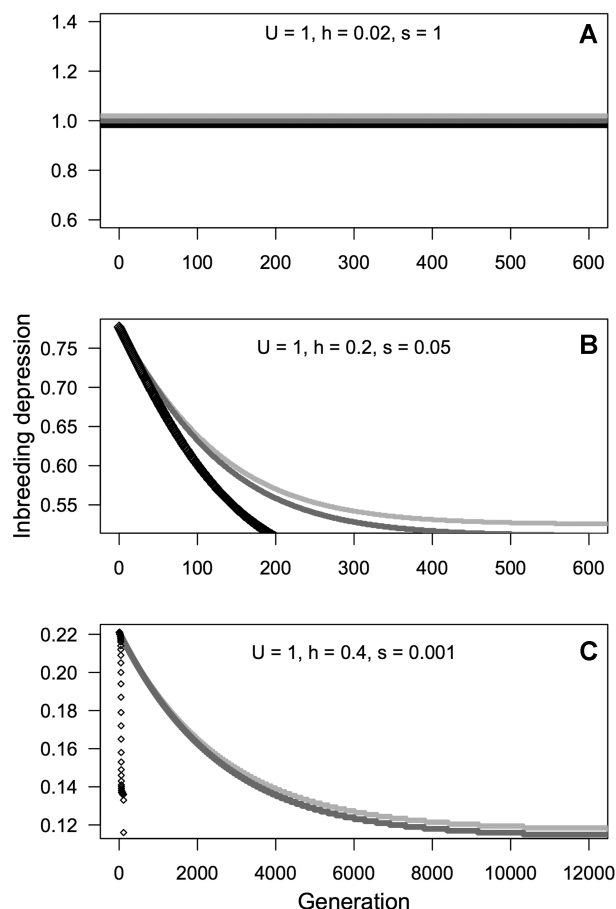


Figure 1. Results from numerical simulation of the evolution of inbreeding depression after a change in mating system for lethal (top), moderately deleterious (middle), and mildly deleterious (bottom) mutations. Each panel shows the expected change in inbreeding depression for a specific combination of genomic mutation rate (U), dominance (h), and selection (s) for a shift from complete outcrossing to selfing frequencies of 0.01 (light gray), 0.05 (dark gray), and 0.80 (black). In the top panel, results are the same for all selfing rates but are displaced slightly for visibility.

simulations supports the prediction that, if most mixed-mating taxa are in evolutionary transition, their mean ID should be less than that for outcrossing taxa.

MATING SYSTEM AND ID: EMPIRICAL RESULTS

We compared the mean of published estimates of ID for mixed-mating, selfing, and outcrossing taxa. We searched the literature for estimates of ID for taxa for which Goodwillie et al. (2005) reported selfing rates for natural populations based on marker analysis of progeny arrays ($N = 345$ species). We included only taxa whose ID estimates were derived from measurements of fitness components from experimental hand self and outcross pollinations.

We excluded studies in which ID estimates were based on declines in the inbreeding coefficient with age, because these do

not include ID expressed as differences in fecundity of selfed and outcrossed progeny (Ritland 1990). We also excluded estimates based on measures of the fitness of selfed and open-pollinated crosses, which can be biased by selfing in the open-pollinated crosses. Finally, we excluded comparisons of progeny from cleistogamous and chasmogamous flowers because these can be biased both by selfing in the chasmogamous flowers and by nongenetic differences in the sizes and germination behaviors of seeds produced by the two flower types (see, e.g., McNamara and Quinn 1977; Lu 2002). For each population, we recorded fitness components for up to four life-cycle stages. We defined the four stages as in Husband and Schemske (1996), as seed production of pollinated flowers, seed germination, juvenile survival, and growth or reproduction of progeny resulting from hand pollinations.

Studies differ in the traits used to estimate ID in the earliest and latest life-cycle stages. For the earliest stage, we recorded seed number per cross when it was available but also accepted proportion fruit set or, in a few cases, seed weight. For the last stage, we recorded seed or fruit production but also accepted flower number. If none of these was reported, we accepted a measure of vegetative size such as biomass, height, or leaf number.

To minimize variation in our estimates of ID caused by differences in the environmental conditions under which fitness was measured (reviewed by Armbruster and Reed 2005), we included estimates for progeny raised in the greenhouse in preference to those measured in the field whenever possible. Estimates of ID from less harsh environments tend to be biased downward (Armbruster and Reed 2005), but because we wished to identify general patterns across many species, we chose the systematic bias possible in greenhouse estimates over the obfuscating noise that could be created by comparison of ID measured in more variable field environments.

For 16 species, including all 10 gymnosperms and the only angiosperm tree (*Eucalyptus regnans*), measurements of ID were from field or garden-grown progeny. In most of these cases, seeds were germinated in the laboratory, and subsequent progeny growth was in an outdoor garden under environmental conditions likely to be less variable and more benign than natural field conditions.

We calculated the fitness of selfed offspring relative to outcrossed offspring for each life-cycle stage and cumulative ID as $(1 - [\text{the product of the relative fitnesses at all stages}])$. We also calculated cumulative ID including only seed set per cross, percentage germination, and growth/fecundity, the three stages for which data are most commonly reported. We distinguish our two estimates of cumulative ID as four-stage ID, which includes juvenile survival, and three-stage ID, which does not.

Before analyses, we converted all measured selfing rates to estimates of primary selfing, proportion of selfing at fertilization,

Table 1. Mean (standard error of the mean) inbreeding depression expressed at each of four successive life-cycle stages for all taxa in our dataset, angiosperms only, gymnosperms only, and gynodioecious taxa only. For all taxa and for angiosperms only, means in the same row that share a superscript do not differ significantly (Mann–Whitney U tests). Sample sizes are for all stages except for survival, for which N is 56 for all taxa, 47 for angiosperms, nine for gymnosperms, and seven for gynodioecious taxa.

	N	Seed set	Germination	Survival to flowering	Growth/reproduction
All taxa	68	0.206 ^{AC} (0.032)	0.116 ^{BC} (0.018)	0.134 ^B (0.032)	0.220 ^A (0.019)
Angiosperms	58	0.143 ^B (0.026)	0.127 ^{BC} (0.019)	0.119 ^C (0.035)	0.226 ^A (0.024)
Gymnosperms	10	0.571 (0.098)	0.053 (0.032)	0.211 (0.089)	0.187 (0.033)
Gynodioecious taxa	10	0.287 (0.073)	0.239 (0.051)	0.234 (0.149)	0.247 (0.065)

by correcting for ID that occurred in life stages before progeny were screened for markers (Maki 1993). We classified cases with primary selfing rates ≤ 0.2 as outcrossing, those with primary selfing rates ≥ 0.8 as selfing, and all cases with primary selfing rates between 0.2 and 0.8 as mixed mating (as in Schemske and Lande 1985). We excluded all cases lacking marker-based estimates of the mating system from our analyses because of the difficulty of distinguishing mixed mating from other categories without this quantitative estimate.

To assess the relationship between ID and selfing, we matched estimates of selfing to measures of ID for the same population whenever possible. For gynodioecious taxa, we calculated the mating system at the population level, except for *Thymus vulgaris*, for which the only estimate available was the selfing rate of hermaphrodites. For two species (*Datura stramonium* and *Phlox drummondii*), no estimate of selfing was available for the population for which ID was measured, but estimates from multiple other populations of each species were available and differed by no more than 0.1. For these species, we averaged selfing across populations. In cases where multiple estimates of either ID or selfing were available for the same population ($N = 20$), we averaged them. When data for both ID and selfing were available for multiple populations of the same species ($N = 15$), we averaged estimates of each whenever primary selfing rates differed by less than 0.2 (the width of the range we chose to define selfing and outcrossing taxa). If selfing estimates differed by 0.2 or more, we included both populations in the analyses.

Description of the data

We located data for 100 populations of 58 species (Table 1, Fig. 2). Pooling data for similar populations of the same species yielded a total of 68 cases. The data are spread fairly consistently across the potential range of both cumulative ID and primary selfing, except that the number of taxa with primary selfing between 0.2 and 0.4 is about twice that in other intervals of the same width (Fig. 2).

The 58 species came from 27 families and included 10 gymnosperm species. Sixteen species were represented by more than one population in the full dataset of 100 populations, and nine

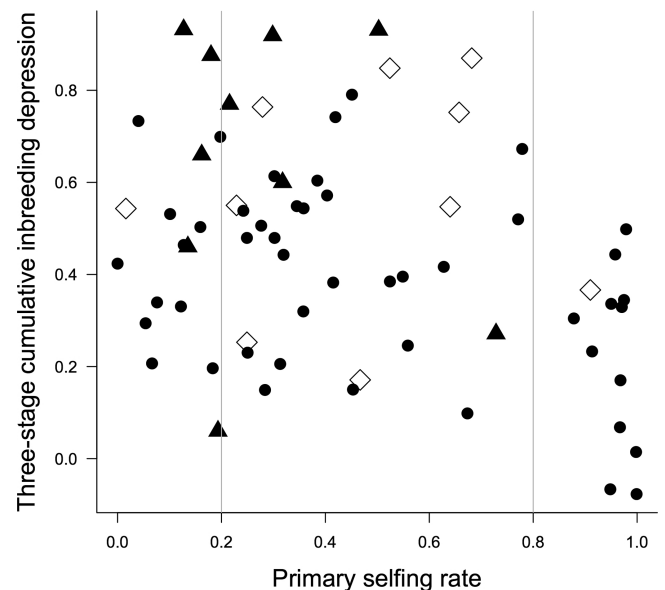


Figure 2. The relationship between three-stage cumulative inbreeding depression and primary selfing rate for 68 populations. Gymnosperms are indicated by triangles and gynodioecious taxa by diamonds. Vertical lines mark the primary selfing-rate cutoffs we chose to define mixed-mating taxa ($0.2 < \text{primary selfing rate} < 0.8$).

were represented by more than two after populations of the same species with similar mating-system estimates were averaged.

ANALYSES

For the full set of 68 cases, we calculated mean cumulative ID for selfing, mixed-mating, and outcrossing cases, treating mating system as a discrete trait. Because sample sizes were modest (13 selfing taxa, 38 mixed-mating taxa, and 17 outcrossing taxa), we took the statistically conservative approach of comparing the locations of the distributions of ID with Mann–Whitney U tests rather than comparing means with parametric tests. We also compared the magnitude of ID expressed at each life-cycle stage averaged for all taxa in the dataset with Mann–Whitney U tests. Finally, we compared the distributions of ID for each life-cycle stage in selfing, mixed-mating, and outcrossing cases with

Mann–Whitney U tests. We held type I error rates at 0.05 for multiple comparison with a sequential Bonferroni procedure (Holm 1979), but doing so did not change the results, so we report only uncorrected significance.

We plotted ID as a function of primary selfing rate to identify mixed-mating taxa with strong ID relative to their selfing rates. We also calculated Spearman's rank correlation to quantify the relationship between primary selfing and both three- and four-stage ID for all cases in the dataset. Because our data included estimates of ID and selfing for multiple populations of some species, we also examined relationships between population-level estimates of selfing and ID for each species for which we had data for more than two populations.

Summary statistics for mating-system estimates

Mean primary selfing for all 68 cases was 0.45, and mean cumulative three- and four-stage ID values were 0.45 and 0.52, respectively. ID was notably greater for gymnosperms than for all other taxa (gymnosperm mean three-stage ID = 0.65, $N = 10$; angiosperm mean = 0.41, $N = 58$).

The average magnitude of ID varied with life-cycle stage. Mean ID was nearly twice as great for the earliest (seed set) and latest (adult growth and reproduction) life-cycle stages as for the intermediate stages (germination and juvenile survival; Table 1), but when only angiosperms were included in the analysis, ID for the earliest stage was more similar to those of the intermediate stages. The mean for the latest stage remained nearly twice as large (Table 1). Exclusion of gymnosperms had a substantial effect on these results because these taxa express relatively strong ID between fertilization and seed maturation and weak ID at germination (Table 1).

Rank correlation between ID and primary selfing was negative for three-stage ID ($\rho = -0.26$, one-tailed $P = 0.015$, $N = 68$) and four-stage ID ($\rho = -0.18$, one-tailed $P = 0.10$, $N = 56$), although the latter was not significant. Removing gymnosperms from the analysis had little effect on the rank correlation between three-stage ID and primary selfing ($\rho = -.24$, one-tailed $P = 0.04$, $N = 58$).

Evidence for evolutionary transition in the mating system

We expected that, if mixed mating reflects evolutionary transition in most taxa with intermediate selfing rates, then the mean ID of mixed-mating taxa should be between the mean for selfers and that for outcrossers. We also expected that ID at the seed stage would be particularly weak for mixed-mating taxa if many are in transition. Our comparisons of overall and stage-specific ID do not support these expectations. The mean three-stage ID was more than twice as large for outcrossers (0.48) as for selfers (0.23), and the mean for mixed-mating taxa (0.51) was slightly, though

not significantly, greater than that for outcrossers (Fig. 3). The distributions of three-stage ID for mixed-mating and outcrossing taxa are similar, and both are shifted significantly upward relative to that of selfing taxa (Fig. 3). Four-stage ID was available for 56 cases, and the pattern and significance of differences among mating-system classes was similar to that for three-stage ID (mean four-stage ID for selfers was 0.26, for mixed-mating taxa 0.58, and for outcrossers 0.54). Removing gymnosperms from these comparisons had little effect; for angiosperms alone, mean three-stage ID was 0.23, 0.48, and 0.44 for selfing, mixed-mating, and outcrossing taxa, respectively. The estimates for mixed-mating and outcrossing taxa are not significantly different from each other, and both are significantly greater than that for selfers (results not shown). For all four stages, mean ID was 0.26, 0.55, and 0.44 for selfing, mixed-mating, and outcrossing angiosperm taxa, respectively.

The magnitude of life-cycle stage-specific expression of ID also differed for selfing, mixed-mating, and outcrossing taxa (Fig. 4). ID expressed by mixed-mating taxa at the seed stage was significantly greater than that of selfers and not significantly different from that of outcrossers. At germination, mating-system classes did not differ significantly in ID. ID for progeny survival to flowering and for adult growth or reproduction was significantly greater for mixed-mating than for selfing taxa. Outcrossers had intermediate ID at both of these stages and were not significantly different from either selfing or mixed-mating taxa.

When gymnosperms, which have exceptionally high early ID (Table 1), were removed from the comparisons of stage-specific ID, differences among mating-system classes in early ID were no longer significant (Fig. 5). For angiosperms alone, selfing and outcrossing taxa did not differ in the expression of ID at seed germination or survival to flowering, but ID at germination was significantly greater for mixed mating than for selfing taxa, and ID in survival to flowering was greater for mixed mating than for either selfing or outcrossing taxa (Fig. 5). Removal of gymnosperms did not affect the contrasts for ID expressed as adult growth or reproduction.

Evidence consistent with selective interference

Of the 10 taxa with the strongest three-stage ID ($ID > 0.75$), eight were mixed mating. Also, of these 10, five were gymnosperms, and four were gynodioecious (Fig. 2). In addition, gynodioecious *Cucurbita foetidissima* had a moderate three-stage ID (0.55), but the four-stage ID for this species (0.87) was in the range of the top 10 estimates (Table 2). The large stature and great longevity of gymnosperms are traits proposed to promote selective interference because they increase per-generation mutation rates (Schofield and Schultz 2006). Both the strong overall ID and the exceptionally strong ID expressed at the seed stage by gymnosperms (Table 1) are expected when selective interference occurs. Of the

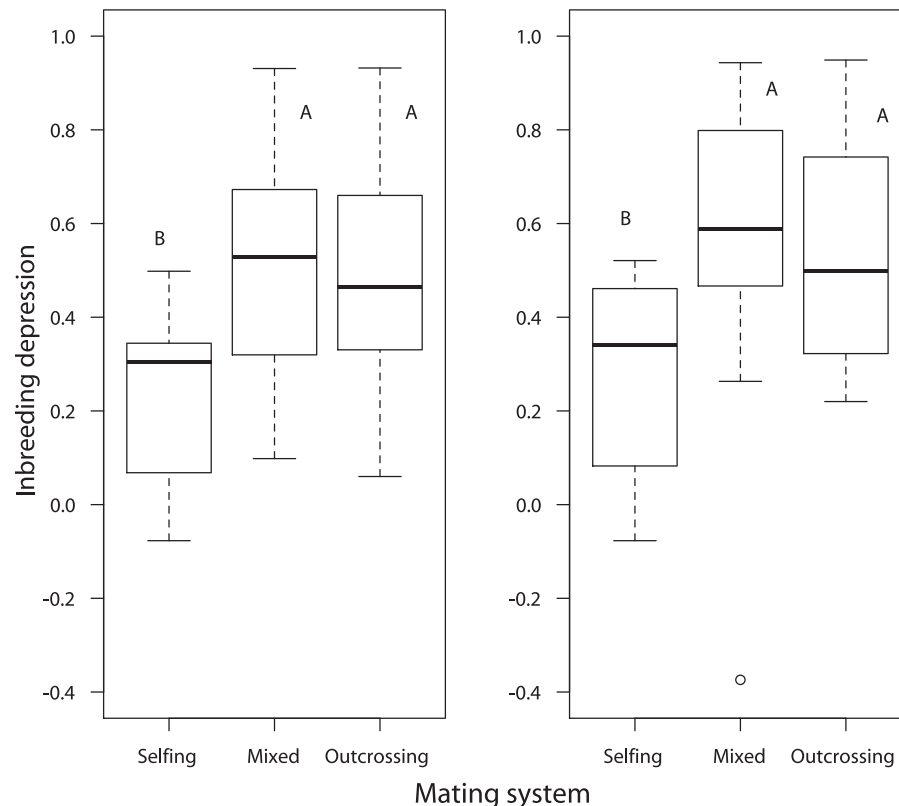


Figure 3. Box plots for three- (left panel) and four-stage (right panel) cumulative inbreeding depression for taxa designated selfing (primary selfing rate > 0.8), mixed mating ($0.2 < \text{primary selfing rate} < 0.8$), and outcrossing (primary selfing rate < 0.2). Box top and bottom indicate upper and lower quartiles of the distribution. Vertical lines show $1.5 \times$ the interquartile range; points that fall beyond this range are indicated individually as open circles. The heavy horizontal bar indicates the median. Distributions that share a letter are not statistically significantly different by Mann–Whitney U test.

gynodioecious taxa with strong ID, *Schiedea salicaria* is woody and possibly long lived, *Wurmbea dioica* is a long-lived perennial, and *C. foetidissima* is a large, clonal perennial. *Bidens sandwicensis* is small and short lived, and *T. vulgaris* is a small shrub.

A mean adult inbreeding coefficient close to zero in mixed-mating populations is a clear signature of selective interference (Lande et al. 1994; Scofield and Schultz 2006). We calculated the mean inbreeding coefficient among parents for selfing, mixed-mating, and outcrossing taxa for cases in our dataset for which it was reported. Data were limited, but the coefficient was close to zero for both mixed-mating taxa (0.038, $N = 12$) and outcrossing taxa (0.006, $N = 6$). Both of these were considerably lower than the mean for selfing taxa (0.545, $N = 8$).

Variation in ID among populations within species

For populations within each of nine species for which data for both ID and selfing were available for more than two populations, the relationships between ID and selfing ranged from positive to negative (Fig. 6). For *Leptosiphon jepsonii*, *Eichhornia paniculata*, and *Clarkia tembloriensis*, ID declined consistently as selfing increased, as would be expected if within-species trends mirror the

relationship in the full dataset. The relationship between ID and selfing was also negative for *Collinsia heterophylla* and *C. verna*, but a sign test indicated that the observed frequency of species with negative relationships (six out of nine) was not higher than expected by chance ($P = 0.25$). ID and selfing appeared unrelated for populations of *Mimulus guttatus*, *Salvia pratensis*, *Chamerion angustifolium*, and *Lupinus perennis*, but we lack data from either highly selfing or highly outcrossing populations of these species, which would provide the best contrast for detecting a relationship between the mating system and ID.

Discussion

We found that the average ID expressed by mixed-mating taxa is as strong as that of highly outcrossing taxa. Such strong ID indicates that not all mixed mating can be dismissed as a transitory phase in evolution toward a more extreme mating system. Strong cumulative ID; strong expression of predispersal ID in large, long-lived taxa; and an average inbreeding coefficient for parents that is close to zero all support the potential for selective interference to contribute to stable mixed mating in self-compatible taxa that

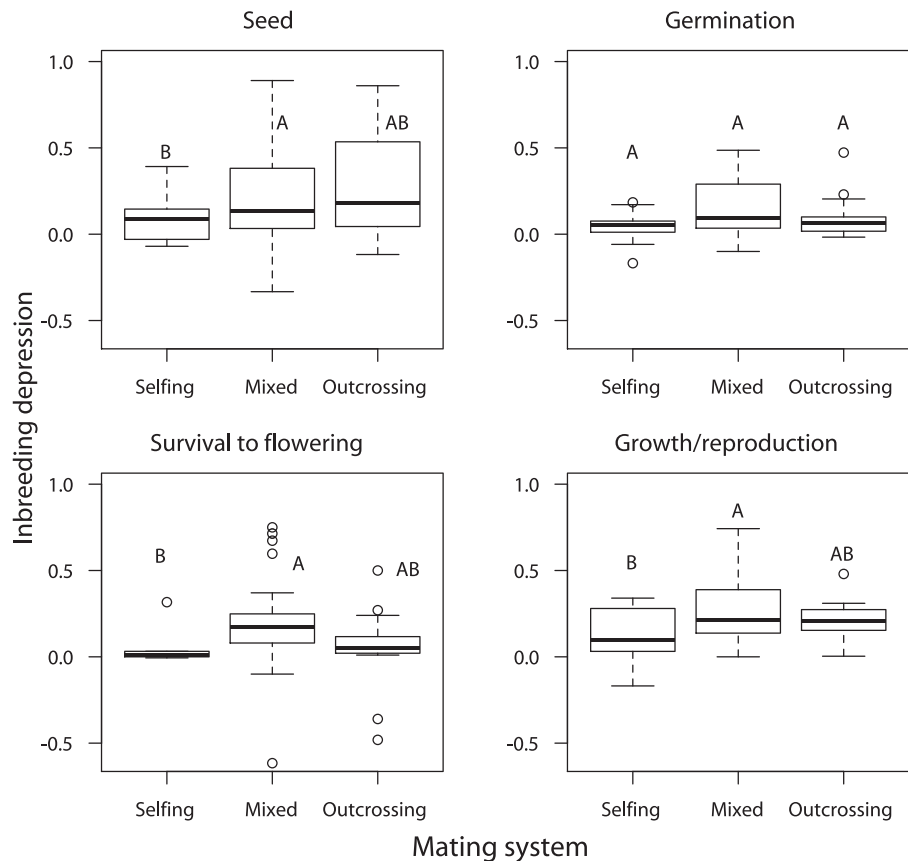


Figure 4. Inbreeding depression expressed at each of four life-cycle stages for selfing, mixed-mating, and outcrossing taxa for all 68 taxa included in the quantitative analyses. Box top and bottom indicate upper and lower quartiles of the distribution. Vertical lines show $1.5 \times$ the interquartile range; points that fall beyond this range are indicated individually as open circles. The heavy horizontal bar indicates the median. Distributions within a stage that share a letter do not differ significantly by Mann-Whitney U test.

cannot prevent the receipt of self-pollen. Beyond the inferences supported by our analyses, we conclude that, despite longstanding interest in the puzzle of mixed mating, our insight into what explains its occurrence remains limited by the quantity and quality of available empirical data.

We interpret strong average ID for mixed-mating taxa as evidence that mixed mating can be maintained in some populations in spite of strong ID. An alternative is that error in the estimation of selfing caused us to classify some highly outcrossing taxa as mixed mating and therefore to overestimated ID in mixed-mating taxa. Estimates of mating system based on small numbers of polymorphic markers can overestimate selfing because there is little power to distinguish selfing from biparental inbreeding. We believe this bias had little influence on our results because, for cases in which we have information on the number of markers used, mating-system estimates for mixed-mating taxa were based on an average of 3.4 markers per population, and the relationship between number of markers and estimated selfing for mixed-mating cases is weak and not significantly different from zero ($\rho = -0.153$, $P = 0.44$, $N = 27$; Appendix B).

One other factor that could contribute to high average ID of mixed-mating taxa is overdominance in the expression of ID, which can result in an increase in ID with selfing (Charlesworth and Charlesworth 1987). Several models suggest that, when overdominance underlies ID, the negative consequences of increased selfing caused by increased ID can make mixed-mating stable (e.g., see Maynard Smith 1977; Charlesworth and Charlesworth 1990; Uyenoyama and Waller 1991; Latta and Ritland 1993). Although there is little empirical evidence supporting ID caused by overdominance, the difficulty of demonstrating this phenomenon (reviewed by Charlesworth and Willis 2009) makes it premature to rule out the possibility.

SELECTIVE INTERFERENCE

Equally strong average ID for mixed-mating and outcrossing taxa suggests that at least some of the former carry considerable ID that they have not purged. ID approached a value of 1 for some taxa with selfing rates as high as 0.7 (Fig. 2), suggesting that even substantial selfing may not be sufficient to purge genetic load. Some mixed-mating taxa in our dataset may have only recently

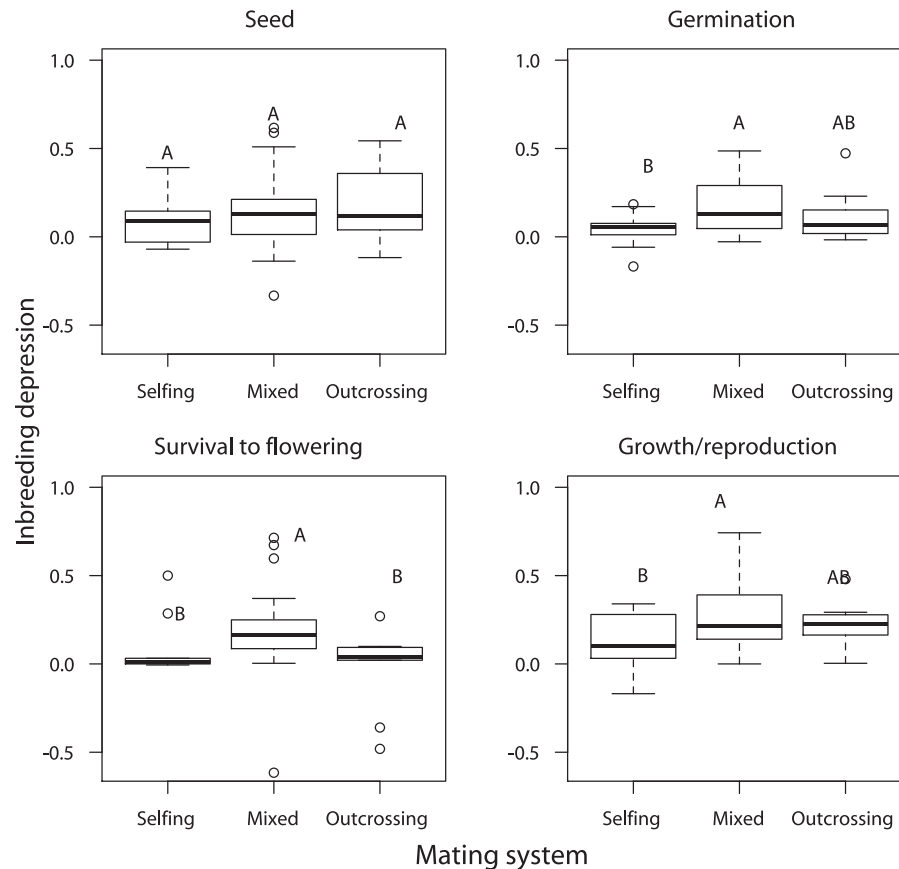


Figure 5. Box plots of inbreeding depression at each of four life-cycle stages for selfing, mixed-mating, and outcrossing taxa for the 58 angiosperm taxa included in quantitative analyses. Box top and bottom indicate upper and lower quartiles of the distribution. Vertical lines show $1.5 \times$ the interquartile range; points that fall beyond this range are indicated individually as open circles. The heavy horizontal bar indicates the median. Distributions within a stage that share a letter do not differ significantly by Mann-Whitney U test.

evolved selfing from an outcrossing ancestor and may be expressing the strong ID that can occur in the early stages of purging, but we argue that it is unlikely that most mixed-mating taxa would fall into this category, especially given the rapid rate of purging suggested by our simulations (Fig. 1). More probably, the large average ID we found reflects the inability of some mixed-mating populations to purge.

Exceptionally strong ID in gymnosperms is consistent with a role for selective interference in preventing purging in these taxa. Greater per-generation mutation rates in long lived, large plant taxa such as trees (Schofield and Schultz 2006) and many clonal species (Vallejo-Marín et al. 2010) are expected to make them more susceptible to selective interference. Our results are consistent with this prediction in that five of our 10 largest estimates of cumulative three-stage ID were for gymnosperm trees, and one was for the long lived, clonal herb *C. angustifolium*. The high average ID expressed by gymnosperms at the earliest life-cycle stage (Table 1) is also consistent with selective interference, which is expected to prevent purging of even early-acting lethal mutations.

Although analysis of progeny arrays from taxa affected by selective interference may indicate mixed mating, if no selfed offspring survive to reproduce, then mating is effectively outcrossing (Lande et al. 1994; Schofield and Schultz 2006). The occurrence of self-fertilization in these taxa is therefore not adaptive but results from the inability to prevent self-pollen from arriving at receptive stigmas, and the absence of effective mechanisms of self-incompatibility. Because the primary selfing rates of taxa exhibiting selective interference can fall within the range of mixed mating, they may make up an appreciable fraction of that group.

Five gynodioecious species with mixed-mating systems (*Wurmbea*, *Schiedea*, *Cucurbita*, *Bidens*, and *Thymus*) also expressed very strong four-stage ID (Table 2). Most of these taxa express both strong ID and strong selfing (Fig. 2), consistent with models that predict that this combination promotes the invasion of hermaphrodite populations by females (Lloyd 1975; Charlesworth and Charlesworth 1978). Selective interference may prevent purging in the gynodioecious taxa that are large or long lived, including *Wurmbea*, *Schiedea*, and *Cucurbita*, but *Bidens* and *Thymus* are both of small stature, and *Bidens* is short lived.

Table 2. Fitness of selfed relative to outcrossed progeny for four life-cycle stages, cumulative inbreeding depression for three and four stages, and primary selfing rate for taxa included in analyses. Four-stage cumulative inbreeding depression includes all four life-cycle stages. Three-stage inbreeding depression does not include survival to flowering. Species are represented by more than one population if selfing rates differ by more than 0.2.

Species	Family	Relative fitness				Inbreeding depression			References
		Seeds	Germ.	Surv.	Fecun.	Three-stage	Four-stage	Selfing	
<i>Abies procera</i> ^G	Pinaceae	0.710	1.000	0.883	0.760	0.460	0.523	0.135	Sorenson (1999) ^{a,b}
<i>Allium schoenoprasum</i>	Liliaceae	0.465	0.915	0.943	0.708	0.699	0.716	0.198	Stevens and Bougourd (1988) ^{a,b}
<i>Amsinckia douglasiana</i>	Boraginaceae	0.872	0.953	NA	0.594	0.506	NA	0.276	Johnston and Schoen (1996), ^b Cheptou and Schoen (2003) ^a
<i>Amsinckia gloriosa</i>	Boraginaceae	1.031	1.000	0.954	0.014	0.014	0.014	0.998	Johnston and Schoen (1996) ^{a,b}
<i>Amsinckia spectabilis</i>	Boraginaceae	1.050	1.059	1.000	0.968	-0.077	-0.077	0.999	Johnston and Schoen (1996) ^{a,b}
<i>Aquilegia caerulea</i>	Ranunculaceae	0.890	0.790	0.800	0.860	0.395	0.516	0.549	Montalvo (1994), ^a Brunet and Eckert (1998) ^b
<i>Begonia hirsuta</i>	Begoniaceae	0.854	0.979	1.006	0.783	0.344	0.340	0.975	Ågren and Schemske (1993) ^{a,b}
<i>Begonia semiovata</i>	Begoniaceae	0.882	0.946	0.968	0.667	0.443	0.461	0.958	Ågren and Schemske (1993) ^{a,b}
<i>Bidens sandwicensis</i> ^D	Asteraceae	0.798	0.514	0.403	0.371	0.848	0.939	0.524	Sun and Ganders (1988), ^b Schultz and Ganders (1996) ^a
<i>Campanula americana</i>	Campanulaceae	0.951	0.978	0.907	0.853	0.207	0.281	0.066	Galloway et al. (2003) ^{a,b}
<i>Chamaecrista fasciculata</i>	Fabaceae	0.860	0.910	0.890	0.590	0.538	0.589	0.242	Fenster (1991), ^a (1995) ^b ,
<i>Chamerion angustifolium</i>	Onagraceae	0.411	0.854	0.846	0.597	0.791	0.823	0.451	Husband and Schemske (1995), ^{a,b} (1997), ^a Parker et al. (1995) ^{a,b}
<i>Chionographis japonica</i> ^D	Liliaceae	0.849	0.830	NA	0.901	0.366	NA	0.910	Maki (1993) ^{a,b}
<i>Clarkia tembloriensis</i>	Onagraceae	0.993	1.010	0.882	0.258	0.742	0.772	0.419	Holtsford and Ellstrand (1989), ^b (1990) ^a
<i>Clarkia tembloriensis</i>	Onagraceae	0.872	0.970	0.727	0.568	0.520	0.651	0.771	Holtsford and Ellstrand (1989), ^b (1990) ^a
<i>Clarkia tembloriensis</i>	Onagraceae	1.061	0.924	0.714	0.684	0.329	0.521	0.971	Holtsford and Ellstrand (1989), ^b (1990) ^a
<i>Collinsia heterophylla</i>	Scrophulariaceae	0.902	1.028	1.616	0.917	0.150	-0.374	0.453	Charlesworth and Mayer (1995), ^b Mayer et al. (1996) ^a
<i>Collinsia heterophylla</i>	Scrophulariaceae	1.138	0.906	NA	0.875	0.098	NA	0.673	Charlesworth and Mayer (1995), ^b Mayer et al. (1996) ^a

Continued.

Table 2. Continued.

Species	Family	Relative fitness				Inbreeding depression			References
		Seeds	Germ.	Surv.	Fecun.	Three-stage	Four-stage	Selfing	
<i>Collinsia verna</i>	Scrophulariaceae	0.956	1.017	0.979	0.726	0.294	0.309	0.054	Kalisz (1989), ^a Kalisz et al. (2004), ^{a,b} S. Kalisz, unpubl. data ^a
<i>Collinsia verna</i>	Scrophulariaceae	0.966	0.983	0.971	0.847	0.196	0.220	0.183	Kalisz (1989), ^a Kalisz et al. (2004), ^{a,b} S. Kalisz, unpubl. data ^a
<i>Crepis sancta</i>	Asteraceae	0.858	0.937	0.901	0.717	0.423	0.481	0	Cheptou et al. (2001), ^a (2002) ^b
<i>Cucurbita foetidissima</i> ^P	Cucurbitaceae	0.660	0.680	0.286	1.000	0.547	0.871	0.640	Kohn (1988), ^a Kohn and Biardi (1995) ^b
<i>Datura stramonium</i>	Solanaceae	0.608	1.168	NA	1.169	0.170	NA	0.968	Motten and Antonovics (1992), ^b Núñez-Farfán et al. (1996), ^a Motten and Stone (2000) ^b
<i>Decodon verticillatus</i>	Lythraceae	0.867	0.837	0.957	0.768	0.443	0.467	0.320	Eckert and Barrett (1994), ^a Eckert (2002) ^b
<i>Eichhornia paniculata</i>	Pontederiaceae	0.933	0.940	0.977	0.860	0.246	0.263	0.559	Toppings (1989), ^a Barrett and Husband (1990) ^b
<i>Eichhornia paniculata</i>	Pontederiaceae	0.905	0.980	0.730	0.745	0.339	0.518	0.076	Toppings (1989), ^a Barrett and Husband (1990) ^b
<i>Eichhornia paniculata</i>	Pontederiaceae	1.030	0.950	0.500	1.090	−0.067	0.467	0.948	Toppings (1989), ^a Barrett and Husband (1990) ^b
<i>Eucalyptus regnans</i>	Myrtaceae	1.333	0.535	0.327	0.863	0.385	0.799	0.524	Eldridge and Griffin (1983), ^a Brown et al. (1985), ^b Frupp et al. (1987), ^b Griffin et al. (1987) ^b
<i>Hydrophyllum appendiculatum</i>	Boraginaceae	0.998	0.923	0.676	0.836	0.230	0.480	0.250	Wolfe and Shore (1992), ^b Wolfe (1993) ^a
<i>Ipomoea purpurea</i>	Convolvulaceae	1.000	0.945	0.900	0.841	0.206	0.285	0.313	Ennos (1981), ^b Chang and Rausher (1999) ^a
<i>Leavenworthia crassa</i>	Brassicaceae	0.820	0.850	NA	0.720	0.498	NA	0.979	Lyons and Antonovics (1991), ^b Charlesworth et al. (1994) ^a
<i>Leptosiphon jepsonii</i>	Polemoniaceae	0.905	0.941	1.006	0.900	0.233	0.228	0.913	Goodwillie (2000), ^{a,b} Goodwillie and Knight (2006) ^a

Continued.

Table 2. Continued.

Species	Family	Relative fitness				Inbreeding depression				References
		Seeds	Germ.	Surv.	Fecun.	Three-stage	Four-stage	Selfing		
<i>Leptosiphon jepsonii</i>	Polemoniaceae	0.906	0.985	0.997	0.654	0.417	0.419	0.628	Goodwillie (2000), ^{a,b} Goodwillie and Knight (2006) ^a	
<i>Leptosiphon jepsonii</i>	Polemoniaceae	0.805	0.975	0.807	0.787	0.382	0.501	0.415	Goodwillie (2000), ^{a,b} Goodwillie and Knight (2006) ^a	
<i>Linanthus bicolor</i>	Polemoniaceae	0.961	0.989	0.985	0.981	0.068	0.083	0.967	Goodwillie (2000) ^{a,b}	
<i>Lobelia cardinalis</i>	Campanulaceae	1.019	0.838	0.957	0.609	0.479	0.502	0.302	Johnston (1990), ^{b+} (1992) ^a	
<i>Ludwigia peploides glabrescens</i>	Onagraceae	0.913	0.816	NA	0.934	0.305	NA	0.878	Flores (1990) ^{a,b}	
<i>Lupinus arboreus</i>	Fabaceae	0.509	0.986	0.906	0.910	0.544	0.587	0.358	Kittelson and Maron (2000) ^{a,b}	
<i>Lupinus bicolor</i>	Fabaceae	1.070	0.940	0.990	0.660	0.336	0.343	0.950	Harding et al. (1974), ^b Karoly (1991) ^a	
<i>Lupinus nanus apricus</i>	Fabaceae	0.770	0.950	0.960	0.930	0.320	0.347	0.358	Harding (1970), ^b Harding et al. (1974), ^b Karoly (1991), ^a (1992) ^b	
<i>Lupinus nanus latifolius</i>	Fabaceae	0.570	0.900	0.950	0.520	0.733	0.747	0.040	Harding et al. (1974), ^b Karoly (1991) ^a	
<i>Lupinus perennis</i>	Fabaceae	0.713	0.934	0.979	0.805	0.464	0.475	0.127	Shi (2004), ^{a,b} Michaels et al. (2008) ^a	
<i>Lupinus perennis</i>	Fabaceae	0.554	0.946	0.907	0.817	0.572	0.611	0.403	Shi (2004), ^{a,b} Michaels et al. (2008) ^a	
<i>Mimulus guttatus</i>	Scrophulariaceae	0.997	0.861	0.825	0.607	0.479	0.570	0.249	Ritland and Ganders (1987) ^b , Ritland (1989), ^b (1990), ^b Dudash and Ritland (1991), ^b Dole and Ritland (1993), ^b Willis (1993), ^{a,b} Leclerc-Potvin and Ritland (1994), ^b Carr and Dudash (1995), ^a M. R. Dudash, unpubl. data cited by Husband and Schemske (1996), ^a Dudash and Carr (1998) ^b	

Continued.

Table 2. Continued.

Species	Family	Relative fitness				Inbreeding depression			References
		Seeds	Germ.	Surv.	Fecun.	Three-stage	Four-stage	Selfing	
<i>Pachycereus pringlei</i> ^p	Cactaceae	0.457	1.004	1.481	0.997	0.543	0.322	0.016	Levin (1991), ^a Murawski et al. (1994), ^b Molina-Freamer et al. (2003) ^{a,b}
<i>Phlox drummondii</i>	Polemoniaceae	0.820	0.770	1.360	0.790	0.503	0.324	0.159	Levin and Bulinska-Radomska (1988), ^a Watkins and Levin (1990) ^b
<i>Picea glauca</i> ^G	Pinaceae	0.130	0.916	0.742	0.656	0.932	0.949	0.127	Fowler and Park (1983), ^a Cheliak et al. (1985b), ^b Innes and Ringius (1990) ^b
<i>Picea mariana</i> ^G	Pinaceae	0.450	0.930	0.760	0.810	0.660	0.742	0.162	Park and Fowler (1984), ^a Boyle and Morgenstern (1986), ^b Sproule (as cited Barrett et al. (1987) ^b
<i>Pinus banksiana</i> ^G	Pinaceae	0.436	0.980	0.727	0.946	0.595	0.706	0.318	Fowler (1965b), ^a Cheliak et al. (1985a) ^b
<i>Pinus elliotii</i> ^G	Pinaceae	0.150	0.707	0.907	0.760	0.919	0.927	0.298	Squillace and Kraus (1962), ^b Snyder (1968) ^a
<i>Pinus ponderosa</i> ^G	Pinaceae	0.355	0.940	0.875	0.675	0.774	0.803	0.216	Sorenson and Miles (1974), ^a Mitton et al. (1981), ^b Farris and Mitton (1984), ^b Sorenson (1999) ^a
<i>Pinus resinosa</i> ^G	Pinaceae	0.986	1.010	NA	1.033	0.006	NA	0.193	Fowler (1962), ^b (1965a), ^{a,b} (1965b) ^b
<i>Pinus taeda</i> ^G	Pinaceae	0.190	0.890	0.500	0.870	0.876	0.938	0.180	Franklin (1972) ^a
<i>Plantago coronopus</i> ^D	Plantaginaceae	0.987	0.965	0.894	0.785	0.253	0.332	0.249	Wolff et al. (1988), ^b Koelewijn (1998) (T) ^a , Koelewijn and van Damme (2005) (ID) ^a
<i>Pseudotsuga menziesii</i> ^G	Pinaceae	0.100	0.960	0.820	0.720	0.931	0.943	0.503	Sorenson (1973), ^b Sorenson and Miles (1974), ^a El-Kassaby et al. (1981), ^b Shaw and Allard (1982), ^b Neale and Adams (1985), ^b Yeh and Morgan (1987), ^b Sorenson (1999) ^a
<i>Sabatia angularis</i>	Gentianaceae	0.967	1.000	0.778	0.880	0.149	0.338	0.284	Dudash and Barrett (1989), ^b Dudash (1990), ^a Husband and Schemske (1996) ^a

Continued.

Table 2. Continued.

Species	Family	Relative fitness				Inbreeding depression			References
		Seeds	Germ.	Surv.	Fecun.	Three-stage	Four-stage	Selfing	
<i>Salvia pratensis</i>	Lamiaceae	0.877	0.714	0.751	0.721	0.548	0.661	0.345	van Treuren et al. (1993), ^b Ouborg and van Treuren (1994) ^a
<i>Schiedea adamantis</i> ^D	Caryophyllaceae	1.075	0.873	NA	0.884	0.171	NA	0.467	Sakai et al. (1997) ^{a,b}
<i>Schiedea lydgatei</i>	Caryophyllaceae	0.857	0.634	0.827	0.730	0.604	0.673	0.385	Norman et al. (1995), ^a (1997) ^b
<i>Schiedea membranacea</i>	Caryophyllaceae	0.886	0.650	0.828	0.671	0.614	0.680	0.302	Culley et al. (1999) ^{a,b}
<i>Schiedea menziesii</i>	Caryophyllaceae	0.788	0.557	NA	0.840	0.673	NA	0.779	Rankin et al. (2002) ^{a,b}
<i>Schiedea salicaria</i> ^D	Caryophyllaceae	0.618	0.710	0.750	0.565	0.752	0.814	0.657	Sakai et al. (1989), ^a Norman et al. (1997) ^b
<i>Silene virginica</i>	Caryophyllaceae	1.118	0.796	NA	0.753	0.331	NA	0.122	Dudash and Fenster (2001) ^{a,b}
<i>Silene vulgaris</i> ^D	Caryophyllaceae	0.815	0.690	0.920	0.800	0.550	0.586	0.229	D. Charlesworth pers. comm. ^{a,b}
<i>Thuja plicata</i> ^G	Cupressaceae	0.720	1.100	0.984	0.921	0.271	0.282	0.729	El-Kassaby et al. (1994), ^b O'Connell et al. (2001), ^b (2004), ^b Russell et al. (2003), ^a J. H. Russell, unpubl. data ^a
<i>Thymus vulgaris</i> ^D	Lamiaceae	0.383	0.554	NA	0.614	0.870	NA	0.682	Valdeyron et al. (1977), ^b Assouad et al. (1978) ^{a,b}
<i>Wurmbea dioica</i> ^D	Colchicaceae	0.491	0.788	0.629	0.611	0.764	0.851	0.279	Ramsey et al. (2006) ^a
<i>Yucca filamentosa</i>	Agavaceae	1.076	0.527	NA	0.826	0.531	NA	0.101	Pellmyr et al. (1997), ^a Huth and Pellmyr (2000) ^a

Germ. = germination; Surv. = survival to flowering; Fecun. = fecundity; Selfing = primary selfing rate.

^GGymnosperms.^DGynodioecious.^aInbreeding depression.^bSelfing rate.

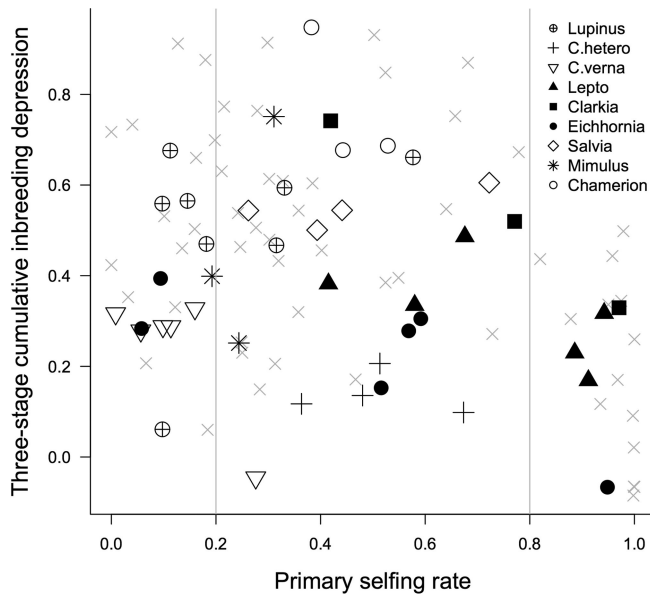


Figure 6. The relationship between three-stage cumulative inbreeding depression and primary selfing rate, highlighting data for sets of populations of the same species. This figure includes all 100 populations for which we found data; multiple populations of the same species were not averaged. Species abbreviations are *Lupinus*, *Lupinus perennis*; *C.hetero*, *Collinsia heterophylla*; *C.verna*, *Collinsia verna*; *Lepto*, *Leptosiphon jepsonii*; *Clarkia*, *Clarkia tembloriensis*; *Eichhornia*, *Eichhornia paniculata*; *Salvia*, *Salvia pratensis*; *Mimulus*, *Mimulus guttatus*; *Chamerion*, *Chamerion angustifolium*.

High rates of population turnover have been proposed to maintain gynodioecy in these taxa (Schultz and Ganders 1996; Thompson and Tarayre 2000) and may also maintain higher rates of ID if populations become locally extinct before the genetic load is purged.

EVIDENCE CONSISTENT WITH EVOLUTION TOWARD SELFING

The negative relationship we observed between selfing and ID among all species and for populations within some species cannot by itself distinguish evolutionary transition in the mating system from mixed mating that is stable at different selfing rates in different populations. For some of the species for which the relationship between selfing rate and ID among populations is consistently negative, other evidence supports the existence of a catalyst that could initiate evolution toward increased selfing: demographic bottleneck associated with colonization accompanied by loss of heterostyly in *E. paniculata* (Ness et al. 2010) and possible selection for selfing as a means of avoiding hybridization with a sympatric congener in *L. jepsonii* (Goodwillie 1999; Weber and Goodwillie 2009). Paleobiogeographic evidence also supports demographic bottlenecks in the two gymnosperm taxa

with low ID, *Pinus resinosa* (Fowler 1965a,b) and *Thuja plicata* (El-Kassaby et al. 1994; O'Connell et al. 2008). Although we cannot rule out stable mixed mating in these taxa, the sum of the evidence in each case supports a scenario of evolution toward greater selfing.

COMPARISON WITH PAST ANALYSES OF ID

In their review of the relationship between ID and mating system, Byers and Waller (1999) found the evidence for purging scant and concluded that it plays a limited role in natural populations. In contrast, our simulations indicated that once selfing ensues in an outcrossing population, rapid purging is expected under a wide range of conditions, and our empirical data showing greater average ID for outcrossing than for selfing taxa are consistent with purging in many natural populations. We also found a significant negative correlation between selfing and ID across all taxa, despite evidence for maintenance of strong ID by selective interference in some mixed-mating taxa, which could obscure this relationship.

Our results are consistent with those of Husband and Schemske (1996), who also concluded that purging is a significant evolutionary force in natural populations. Although we defined selfing and outcrossing mating systems more narrowly than Husband and Schemske (1996), our estimates of mean three-stage ID for primarily selfing and primarily outcrossing taxa (0.23 and 0.44, respectively) are similar to those they reported (0.23 for taxa with primary selfing rates greater than 0.55 and 0.53 for taxa with primary selfing rates less than 0.45).

Our results and those of Husband and Schemske (1996) are based primarily on estimates of ID calculated from the difference in fitness for selfed and outcrossed zygotes from crosses conducted on naturally occurring parental genotypes. This procedure overestimates the genetic load in highly selfing populations, because selfed progeny are more inbred in highly selfing than in highly outcrossing populations. Selfed progeny from a highly inbreeding population will have an inbreeding coefficient close to 1, whereas those from a randomly outcrossing population will have an inbreeding coefficient of 0.5 (Uyenoyama et al. 1993; Charlesworth and Willis 2009). As a consequence, inbred progeny will have greater homozygosity in a selfing population than in an outbred one and will therefore have a greater opportunity to express largely recessive mutations as ID. Because this difference biases against the greater ID in outcrossed taxa reported here and by Husband and Schemske (1996), the actual difference is probably larger.

Although we found substantially greater ID expressed in the earliest and latest life-cycle stages on average, much of the greater ID in the earliest stage was driven by the extreme values for gymnosperms (Table 1; compare Figs. 4 and 5). This pattern could indicate that the strong early ID for primarily outcrossing taxa reported by Husband and Schemske (1996) is not general but

rather is restricted to gymnosperms or to taxa affected by selective interference.

PROSPECTUS

Insights into mixed mating from the relationship between mating system and ID remain limited by the current availability and quality of empirical data. Because only studies that measure ID for all life-cycle stages can be included in comparisons, we urge future empiricists to estimate fitness for all life stages of selfed and outcrossed zygotes. We also urge reporting measures of error as well as means for fitness estimates, to permit the use of meta-analytical techniques, which was not possible for our dataset. More estimates of ID for primarily selfing taxa and primarily outcrossing taxa would increase the power and scope of analyses. Reporting inbreeding coefficients (F_{IS}) would help to identify cases of selective interference because, when this occurs, F_{IS} is expected to be zero even when analysis of progeny arrays indicates appreciable selfing. Inbreeding coefficients were available for less than 40% of the cases in our dataset.

Accurate estimates of ID, which require studies with large samples conducted in natural environments and encompassing the entire life cycle of selfed and outcrossed progeny, are necessary for conclusive inferences about many mechanisms proposed to maintain mixed mating in individual taxa. Many models propose quantitative thresholds for the magnitude and/or age-specific expression of ID necessary to generate selection for a particular mating system (reviewed by Goodwillie et al. 2005; see also Porcher and Lande 2005b; Harder et al. 2008). Currently, such explicit thresholds are difficult to apply because of the large uncertainty associated with estimates of ID arising from the effects of environment on the expression of ID and considerable variation in ID among families within a population.

Not all mechanisms proposed to explain mixed mating produce a distinct pattern of expression of ID (e.g., see Holsinger 1991; Johnston 1998), and a comprehensive explanation for the distribution of mating systems in natural populations must go beyond analysis of ID. Determining whether mixed mating is stable by measuring selection on individuals within populations remains a difficult challenge, but alternative approaches also illuminate the evolution of plant mating systems. Work relating mating system to colonizing ability (e.g., by Baker 1974; Pannell and Dorken 2006; Cheptou and Massol 2009; Randle et al. 2009), selection for dispersal (e.g., see Roze and Rousset 2005), extinction risk (Takebayashi and Morrell 2001), interdemic selection (Pannell and Barrett 1998, 2001; Schoen and Busch 2008), and the balance of selection, drift, and migration in finite populations (Whitlock et al. 2000; Pannell and Barrett 2001; Theodorou and Couvet 2002; Roze and Rousset 2004) has extended our perspective on mating-system evolution to broader spatial and temporal scales. The pronounced evolutionary lability of plant mating systems evi-

denced by wide variation in selfing rates within genera and among populations suggests that phylogenetic correction will not affect the interpretation of broad analyses such as ours. However, relating mating system to ID within clades that include species with mating systems that span the range from selfing to outcrossing could provide deeper insight into the role of ID in mating-system evolution.

Overall, our analyses indicate that despite longstanding interest in mating-system evolution, a wealth of theory, and suggestive patterns in available data, our ability to distinguish among the myriad mechanisms proposed to explain the occurrence of mixed mating in plants remains limited by the availability of empirical data. Future empirical studies linking ID and mating systems across broader spatial and temporal scales, while addressing the critical gaps in the available data described here, will contribute essential evidence necessary to unravel the enigma of mixed mating in plants.

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Appendix A

Results for simulation of purging when selfing was initiated in an outcrossing population for different combinations of mutation rate (U), dominance (h), strength of selection against mutations (s), and frequency of selfing (r). “Initial ID” is the starting strength of inbreeding depression. “Final ID” is the strength of inbreeding depression at equilibrium for the new selfing frequency. “Half life” is the generation at which purging is half complete (i.e., the generation where $ID = 1/2[\text{Initial ID} - \text{Final ID}]$). (A) Results for the parameters in Figure 1. (B) Results for the same parameters as A except that U is reduced to 0.2. (C) Extreme recessivity can result in failure to purge even for moderately to weakly selected mutations and with a high frequency of selfing. (D) The effect of reducing the dominance of weakly selected mutations (compare

with the last three lines of A) and of larger frequencies of selfing. (E) The effect of reducing the dominance of mutations with moderate effects on fitness (compare with the middle three lines of A).

	<i>U</i>	<i>h</i>	<i>s</i>	<i>r</i>	Initial ID	Final ID	Half life
A	1	0.02	1.0	0.99	1.0	1.0	NA
	1	0.02	1.0	0.95	1.0	1.0	NA
	1	0.02	1.0	0.80	1.0	1.0	NA
	1	0.20	0.05	0.99	0.777	0.524	89
	1	0.20	0.05	0.95	0.777	0.510	90
	1	0.20	0.05	0.80	0.777	0.453	90
	1	0.40	0.001	0.99	0.221	0.117	1778
	1	0.40	0.001	0.95	0.221	0.114	1722
	1	0.40	0.001	0.80	0.221	0	114
B	0.2	0.02	1.0	0.99	0.992	0.904	70
	0.2	0.02	1.0	0.95	0.992	0.868	86
	0.2	0.02	1.0	0.80	0.992	0.435	103
	0.2	0.20	0.05	0.99	0.259	0.137	72
	0.2	0.20	0.05	0.95	0.259	0.129	68
	0.2	0.20	0.05	0.80	0.259	0.102	57
	0.2	0.40	0.001	0.99	0.049	0.025	1556
	0.2	0.40	0.001	0.95	0.049	0.024	1464
	0.2	0.40	0.001	0.80	0.049	0.021	1442
C	1	0.02	0.05	0.99	1.0	1.0	NA
	1	0.02	0.05	0.95	1.0	1.0	NA
	1	0.02	0.05	0.80	1.0	1.0	NA
	1	0.02	0.05	0.50	1.0	1.0	NA
	1	0.02	0.001	0.99	1.0	1.0	NA
	1	0.02	0.001	0.95	1.0	1.0	NA
	1	0.02	0.001	0.80	1.0	1.0	NA
	1	0.02	0.001	0.50	1.0	1.0	NA
D	1	0.30	0.001	0.99	0.487	0.281	2608
	1	0.30	0.001	0.95	0.487	0.274	2508
	1	0.30	0.001	0.90	0.487	0.264	2466
	1	0.30	0.001	0.85	0.487	0.254	1582
	1	0.30	0.001	0.825	0.487	0	115
	1	0.30	0.001	0.80	0.487	0	64
	1	0.30	0.001	0.75	0.487	0	16
	1	0.30	0.001	0.50	0.487	0	16
E	1	0.1	0.05	0.99	0.982	0.863	249
	1	0.1	0.05	0.95	0.982	0.855	262
	1	0.1	0.05	0.80	0.982	0.811	300

Appendix B

The relationship between the number of polymorphic marker loci used to measure selfing rate and the estimated primary selfing rate for 59 taxa for which number of markers was available. Number of markers was averaged when selfing rate was an average for multiple populations of the same species. Symbols distinguish selfing, mixed-mating, and outcrossing taxa.

