

**PURGING OF INBREEDING DEPRESSION WITHIN A POPULATION OF
OXALIS ALPINA (OXALIDACEAE)¹**

JENNIFER J. WEBER^{2,5}, STEPHEN G. WELLER², ANN K. SAKAI², ANNIE NGUYEN²,
NGUYEN D. TAI², CÉSAR A. DOMÍNGUEZ³, AND FRANCISCO E. MOLINA-FREANER⁴

²Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697 USA; ³Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, México City 04510 Distrito Federal México; and ⁴Departamento de Ecología de la Biodiversidad, Estación Regional del Noroeste, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 1354, Hermosillo 83000 Sonora, México

- *Premise of the study:* Variation among individuals in levels of inbreeding depression associated with selfing levels could influence mating system evolution by purging deleterious alleles, but empirical evidence for this association is limited.
- *Methods:* We investigated the association of family-level inbreeding depression and presumed inbreeding history in a tristylous population of *Oxalis alpina* (Oxalidaceae).
- *Key results:* Mid-styled individuals possessed the greatest degree of self-compatibility (SC) and produced more autogamous capsules than short- or long-styled individuals. Offspring of highly self-compatible mid-styled individuals showed reduced inbreeding depression. Mid-styled plants that produced capsules autogamously exhibited reduced stigma-anther separation compared to mid-styled plants that produced no capsules autogamously. Reduced inbreeding depression was not correlated with stigma-anther separation, suggesting that self-compatibility and autogamy evolve before morphological changes in stigma-anther separation.
- *Conclusions:* Purging of inbreeding depression occurred in SC mid-styled maternal families. Low inbreeding depression in SC mid-styled plants may lead to retention of the mid-styled morph in populations, despite the occurrence of higher selfing rates in mid-styled relative to short- or long-styled morphs. Variation among individuals in levels of self-fertilization within populations may lead to associations between inbreeding lineages and lower levels of inbreeding depression, influencing the evolution of mating systems.

Key words: autogamy; heterostyly; inbreeding depression; *Oxalis alpina* (Oxalidaceae); purging; self-incompatibility.

INTRODUCTION

Hermaphroditic plant and animal species are capable of self-fertilization, one of the most severe forms of inbreeding, and understanding the selective costs and benefits of inbreeding vs. outbreeding has generated a long history of empirical and theoretical work (e.g., Darwin, 1876, 1877; Nagylaki, 1976; Lande and Schemske, 1985; Schemske and Lande, 1985; Goodwillie et al., 2005). Despite the automatic transmission advantage of selfing (Nagylaki, 1976; Maynard Smith, 1977; Holsinger et al., 1984) and advantages of selfing when pollinators or mates are unreliable (Baker, 1955; Stebbins, 1957; Holsinger, 1996), very few hermaphroditic species are completely self-fertilizing.

A major disadvantage of self-fertilization may be the expression of inbreeding depression (Charlesworth and Charlesworth, 1987).

Inbreeding depression, the reduction in fitness of progeny derived from selfing relative to progeny produced via cross-fertilization, is an important selective force in the evolution of different mating systems (Charlesworth and Charlesworth, 1987). One explanation for reduced fitness of selfed progeny is partial dominance, when inbreeding depression is caused by expression of deleterious homozygous recessive alleles at fitness loci following self-fertilization (e.g., Charlesworth and Charlesworth, 1987; Dudash and Carr, 1998; Charlesworth and Willis, 2009). With partial dominance, a historically selfing population may exhibit reduced inbreeding depression because of purging, the elimination of recessive, deleterious alleles when they are expressed in homozygotes and exposed to selection (e.g., Crow, 1948; Stebbins, 1957; Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Byers and Waller, 1999). Many empirical studies show reduced inbreeding depression with increasing self-fertilization at the population and species level (e.g., Holtsford and Ellstrand, 1990; Barrett and Charlesworth, 1991; Dole and Ritland, 1993; Weller et al., 2005; Goodwillie and Knight, 2006).

Very few studies have addressed the question of whether family-specific inbreeding depression is correlated with the inbreeding history of those families within a population (in flowering plants: Carr et al., 1997; Mutikainen and Delph, 1998; Chang and Rausher, 1999; Daehler, 1999; Takebayashi and Delph, 2000; Stone and Motten, 2002; in hermaphroditic snails:

¹Manuscript received 4 August 2011; revision accepted 6 March 2012.

The authors thank W. Yang and R. Basile for plant care, K. Ngo, Y. Vo, N. Nguyen, M. Tran, M. H. Tran, A. Marcantonio, D. Khuu, M. Nguyen, and M. Ku for help in the greenhouse, first Gustavo Escobedo (INAOE) for access to Sierra La Mariquita. Support was provided by the University of California Institute for Mexico and the United States (UC MEXUS), CONACYT (47858-Q), Universidad Nacional Autónoma de México (PAPIIT IN217803), National Science Foundation grant DEB-0614164 (S. G. Weller and A. K. Sakai, co-PIs), a GAANN fellowship and federal work study funds to J. J. Weber, and Undergraduate Research Opportunity Awards from the University of California Irvine to N. Tai and A. Nguyen. Three anonymous reviewers provided helpful comments on the manuscript.

⁵Author for correspondence (email: jjweber@uci.edu)

Escobar et al., 2009). Significant variation in inbreeding depression among families has been demonstrated in many empirical studies (e.g., Sakai et al., 1989; Carr et al., 1997; Rankin et al., 2002; Pico et al., 2004; Oakley and Winn, 2008; Busch et al., 2010). Families may give rise to lineages where individuals are related by descent. When selfing has a genetic basis, lineages within a population may have different histories of inbreeding if these mating system modifiers have differentially impacted the relative fitness of selfed to outcrossed offspring among lineages (Stone and Motten, 2002; Uyenoyama et al., 1993). Variation among individuals in traits that influence the mating system may increase the potential for associations to develop between inbreeding depression and inbreeding history (Takebayashi and Delph, 2000; Uyenoyama and Waller, 1991a, b, c). We investigated the potential for family-specific inbreeding depression in a predominantly self-incompatible heterostylous species with variation among individuals in traits influencing the mating system.

Heterostylous breeding systems exhibit a reciprocal arrangement of male and female organs within a flower (Barrett et al., 2000), and two or three floral morphs may occur in a population. In flowers of tristylous populations, floral organs occur at three levels. Floral morphs are identified by the level of the stigmas (short-, mid-, and long-styled morphs); for example, short-styled morphs have stigmas at the lowest level and anthers at the mid and highest levels. In typical heterostylous species, pollinations between anthers and stigmas of different levels, including self-pollinations, are not capable of producing offspring (Darwin, 1877). This self-incompatibility prevents self-fertilization and subsequent expression of inbreeding depression, and impacts the maternal component of fitness (Barrett and Shore, 2008). Reciprocal herkogamy (spatial separation of stigmas and anthers) functions to promote cross-pollination between style morphs because pollinators are likely to pick up pollen on different

parts of their bodies and transfer pollen to stigmas at those same heights on flowers of a different style morph (Darwin, 1877; Kohn and Barrett, 1992; Lloyd and Webb, 1992). Reciprocal herkogamy should reduce pollen wastage on incompatible stigmas and increase male fitness.

In tristylous species, a change from self-incompatibility to self-compatibility (SC) of the mid-styled morph may be especially likely because unlike the short- and long-styled morphs, the mid-styled morph has two anther whorls adjacent to the stigmas (Charlesworth, 1979; Fig. 1). Self-compatibility and self-pollen deposition may be associated with proximity of anthers to stigmas, and both are more likely to occur in mid-styled plants (Charlesworth, 1979; Fig. 1). In several heterostylous taxa the mid-styled morph exhibits weak self-incompatibility and has morphological modifications that may promote self-fertilization (*Lythrum* (Lythraceae) Stout, 1925; *Oxalis* (Oxalidaceae) Ornduff, 1972; and *Eichhornia* (Pontederiaceae) Barrett, 1988).

Selfing lineages may have the potential to evolve in tristylous populations because of the greater likelihood of self-fertilization of the mid-styled morph relative to the other two floral morphs. The genetic basis of inheritance of style morphs is known in at least two tristylous plant families (Oxalidaceae: Weller, 1976b; Lythraceae: Eckert and Barrett, 1993; Gettys and Wofford, 2008). In *Oxalis* (section *Ionoxalis*), expression of floral morphs is controlled by a two-locus system, with one locus controlling the expression of the short-styled vs. non-short-styled phenotype (*S-locus*) and the second locus (*M-locus*) controlling the expression of the mid-styled vs. long-styled phenotype; the short allele is epistatic to the mid/long locus (Weller, 1976b). Despite some variation in mode of inheritance among tristylous species, after a few generations of self-fertilization, parents and offspring will generally have identical style morphs. If offspring inherit mating systems modifiers influencing

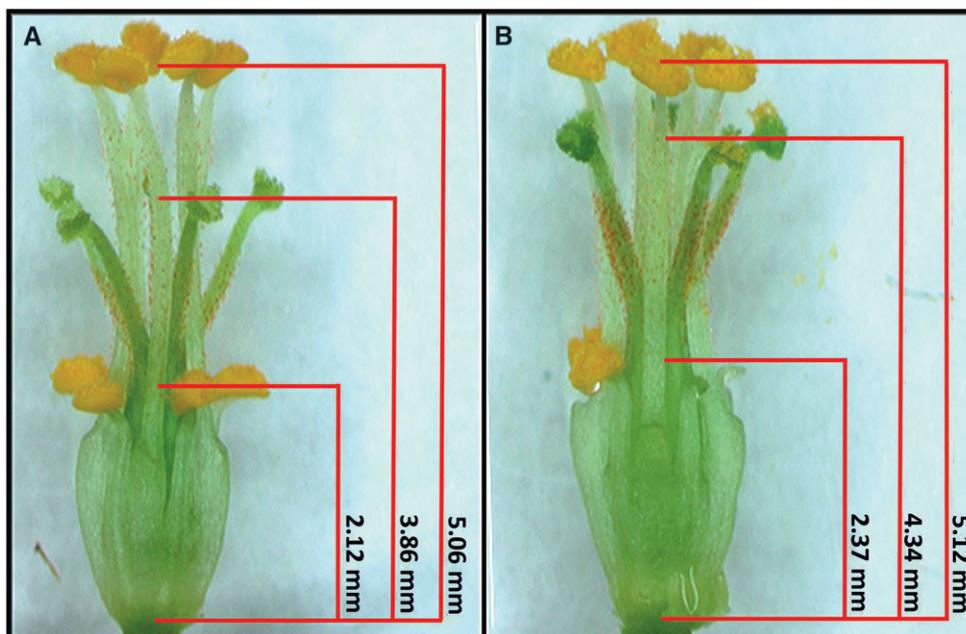


Fig. 1. Floral measurements of mid-styled individuals to quantify convergence in length between mid-level stigmas and long-level anthers (see text for calculation of the convergence index). As the length of the mid-styled stigmas converge to the same position as the long-level anthers, this index approaches one. A, Typical mid-styled morph, convergence index = 0.76. B, Mid-styled individual with more convergent stigmas and long-level anthers, convergence index = 0.87.

traits such as SC or reduced herkogamy, self-fertilizing lineages could evolve within heterostylous populations. A history of self-fertilization in mid-styled lineages could lead to purging of deleterious mutations, reducing the cost of selfing in these lineages and leading to evolutionary modifications of tristylity.

We examined the relationship between family-level inbreeding depression and prior inbreeding history in a population of *Oxalis alpina* (Oxalidaceae section *Ionoxalis*), a heterostylous species where self-compatibility has evolved in some individuals. This species exhibits considerable variation among populations in breeding system traits, including the presence of self-compatibility (Weller et al., 2007), and the ability to form capsules autogamously, i.e., through self-fertilization within the flower without the aid of pollinators. We addressed five questions: (1) How does style morph affect the likelihood of autogamy?; (2) Do individuals with high SC exhibit increased ability to produce capsules autogamously? The evolution of autogamy in association with SC in some individuals could strongly impact the mating system; (3) Are high SC and/or autogamy associated with decreased stigma-anther separation? Morphological changes that result in self-pollen deposition are likely to evolve after the evolution of SC; (4) Do families of individuals with high levels of SC, high levels of autogamy, or reduced stigma-anther separation exhibit reduced inbreeding depression in offspring relative to other families in the same population? Reduced inbreeding depression in the offspring of individuals exhibiting traits that may facilitate self-fertilization would provide evidence of purging and; (5) How does variation in family-level inbreeding depression potentially impact the evolution of distyly from tristylity?

MATERIALS AND METHODS

Study species and population—*Oxalis alpina* (Rose) Knuth (section *Ionoxalis*: Oxalidaceae) ranges from Guatemala to the southwestern United States (Denton, 1973); this perennial species grows from bulbs in response to summer rains. Populations are either distylous or tristylous (Weller, 1976a; Weller et al., 2007). We focused on a tristylous population from Sierra La Mariquita (31°02'46" N, 110°23'20" W, 2404 m), an isolated mountain range in northeastern Sonora, Mexico (voucher: *Weller and Sakai 960*, US). Populations of *O. alpina* from this region are tetraploid (Weller and Denton, 1976). The Sierra La Mariquita population exhibits a typical tristylous self-incompatibility system in which fertilizations involving pollen from anthers and stigmas of the same level in different floral morphs result in greater seed set than all other types of fertilization (Weller et al., 2007). Seed production per capsule of naturally pollinated plants of this population did not vary widely among style morphs. In 2006, field seed production per capsule (given as mean \pm SE, sample size, throughout) from naturally pollinated plants had values of 17.0 ± 1.0 , $n = 57$ plants; 17.0 ± 1.0 , $n = 49$ plants, and 16.4 ± 1.0 , $n = 77$ plants for short-, mid-, and long-styled plants, respectively; field values in 2007 were 14.1 ± 0.9 , $n = 83$ plants; 15.2 ± 0.9 , $n = 75$ plants and 15.2 ± 0.8 , $n = 96$ plants for short-, mid-, and long-styled plants, respectively. Despite the high level of self-incompatibility at the population level, some individuals of this population were partially self-compatible and produced many viable seeds when self-fertilized (Weller et al., 2007). In particular, some mid-styled individuals exhibited high levels of SC when self-pollinated with pollen from the long-anther whorl (Weller et al., 2007). Individuals used in greenhouse crosses in the current study were taken from throughout the population to minimize the potential for sampling related individuals growing near one another (Weller et al., 2007).

Frequency of autogamous plants—Using a pollinator-free greenhouse, we assessed the occurrence of autogamy (production of capsules via self-fertilization in the absence of pollinators) in all three floral morphs of the Sierra La Mariquita population. Our sample size of short-, mid-, and long-styled individuals in the greenhouse was 24, 16, and 30 individuals, respectively. Style-morph frequencies for Sierra La Mariquita are isoplethic in the field, but

mortality in the greenhouse decreased the number of mid-styled individuals in the greenhouse. We presume this mortality occurred randomly with respect to features related to self-compatibility and autogamy. Because flowering plants from this population were collected in the field without respect to autogamy, SC, or style morph, we assume that our greenhouse collection represents a sample of the natural variation in the field.

Assessment of self-compatibility and autogamy based on seed production—Self-compatibility of mid-styled plants was based on the number of seeds per pollination following hand self-pollination using pollen from the long-anther whorl, following the methods of Weller et al. (2007). We included all healthy mid-styled individuals sampled from the field population ($n = 16$). For short- and long-styled morphs, we included five individuals each out of 24 short- and 30 long-styled individuals, deliberately including the four short-styled individuals that produced capsules autogamously in the greenhouse (no long-styled individuals produced capsules autogamously). We included short-styled individuals with autogamous fruit production to increase the potential to examine associations between inbreeding depression and inferred inbreeding history for short- as well as mid-styled morphs. Three different pollination treatments (self, cross, and autogamous) were analyzed for these 26 individuals: (1) Self-pollinations were made by applying self pollen from the same flower ($n = 735$ pollinations; 11.7 ± 2.1 capsules/plant, $n = 26$ plants); (2) Cross-pollinations ($n = 664$ pollinations; 25.0 ± 1.8 capsules/plant, $n = 26$ plants) were used to assess the level of cross-compatibility of maternal plants. Floral morphs were crossed to three to five different paternal parents of different floral morphs using pollinations that typically produce seeds in tristylous populations (from anthers and stigmas of the same level). We used fine forceps to transfer pollen; forceps were washed with ethanol after pollination to prevent pollen contamination. Ripe capsules were collected 14–15 d after pollination before explosive dehiscence and; (3) To measure seed production of autogamous plants, we collected all ripe capsules formed without hand-pollination and counted the number of seeds per capsule ($n = 275$ capsules collected; 23.3 ± 12.5 capsules/plant, $n = 11$ autogamous plants, i.e., plants capable of producing capsules autogamously).

In a separate set of measurements, we assessed the frequency of capsules produced without pollination on these 26 experimental plants. We haphazardly tagged unpollinated flowers (mean [range] = 27 [11–42]) flowers tagged for each of the 26 genotypes. Because the few short-styled plants with autogamy were deliberately sampled, our estimate of differences in the frequency of autogamous seed production between mid- and short-styled plants is conservative. For the capsules that formed on tagged flowers, we counted seed production per capsule (for the 8 out of 26 plants that produced capsules).

Seed production in 26 individuals was compared using an ANOVA with pollination treatment (self, cross, and autogamous) and floral morph (short-, mid-, and long-styled) as fixed effects. For self- and cross-fertilized treatment categories, mean seed production was calculated based on the number of seeds per pollination for each maternal parent. For the autogamy treatment, mean seed production was calculated based on the number of seeds per capsule. Tukey tests were used to compare the number of seeds produced from the three different pollination treatments. To assess the effect of autogamy on seed production of randomly tagged flowers, one sample *t* tests were used to determine whether seed production differed significantly from zero for short-, mid-, and long-styled individuals.

Mid-styled individuals in our experiment produced an average of either more than 10 or fewer than 2.5 seeds per pollination following self-pollination (Fig. 2). We used this natural break in seed production to categorize mid-styled individuals as either with high SC (>10.0 seeds per pollination following self-pollination, $n = 5$ mid-styled individuals) or with low SC (< 2.5 seeds per pollination following self-pollination, $n = 11$ mid-styled individuals). We used a two-tailed Mann-Whitney *U* test to examine whether mid-styled individuals with high levels of SC differed from individuals with low SC in the extent of autogamy (measured by autogamous seed production per capsule).

Stigma-anther convergence—Morphological traits potentially influencing autogamous capsule production of the mid-styled morph were measured in 14 mid-styled individuals. For each of three flowers per individual, petals and sepals were removed to expose the anthers and stigmas; the lengths of all reproductive whorls were measured using a Zeiss Stereo Discovery V8 Zoom dissecting microscope (Fig. 1). Morphological traits of flowers on the same plant measured at two different times (10–14 d apart) were compared using a paired *t* test to determine if morphological traits were consistent through time.

In this population, mid-level stigmas converged only on the level of long-level anthers; convergence was calculated as:

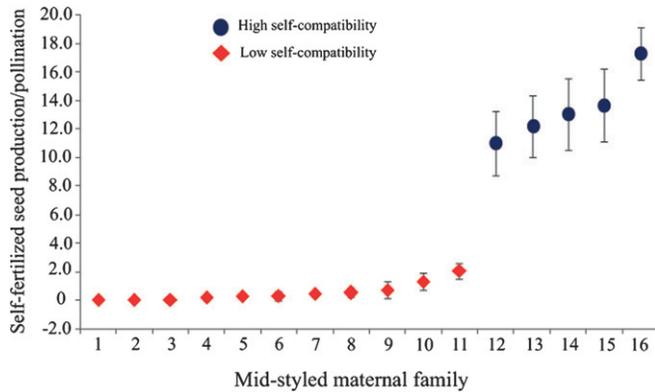


Fig. 2. Mean seed production per pollination (\pm one standard error) in 16 mid-styled plants used to make maternal families. Five maternal plants produced >10 seed per pollination (circles) and 11 mid-styled plants produced <2.5 seeds per pollination (diamonds). These two groups were used to test the effect of SC on the expression of inbreeding depression.

$$\text{Convergence index} = \frac{\text{length of mid-level stigmas}}{\text{length of long-level anthers}}$$

As the length of the mid-level stigmas converge to the same position as the long-level anthers this index approaches one.

A two-tailed Mann-Whitney U test was used to compare the convergence index of high SC individuals ($n = 5$) and low SC individuals ($n = 11$), and the convergence index of autogamous mid-styled individuals (autogamous seed production > 1.0 ; $n = 7$) to nonautogamous mid-styled individuals (autogamous seed production = 0; $n = 9$).

Assessment of inbreeding depression—Progeny from self- and cross-fertilized pollinations (95 ± 1.3 and 491 ± 1.9 seeds planted per maternal family, respectively) were used to assess the level of inbreeding depression within 23 families (inbreeding depression (δ), where $\delta = 1 - w_s/w_o$, and where w_s and w_o are the values of measured traits for selfed and outcrossed progeny, respectively); selfed progeny were not available for families of 3 long-styled maternal plants). Ten seeds were planted per 2-inch pot and pots were placed haphazardly with respect to each other on benches in the University of California, Irvine greenhouses. The proportion of seeds that emerged per pot was calculated after cotyledons appeared.

We transplanted progeny from each maternal individual into 2-inch pots to establish 26 maternal families (mean number of progeny transplanted per maternal family per treatment: Self-fertilized: 17; cross-fertilized: 56). Transplanted seedlings in the cross-fertilized treatment were evenly distributed among three to five different paternal parents per maternal individual. The number of flowers per inflorescence, total number of inflorescences, and total number of flowers were recorded at least weekly for every transplanted individual ($n = 2,097$ individuals) throughout the six-week flowering season. Total biomass (all standing aboveground mass, all bulbs, and roots) was harvested for each plant after dormancy, dried to constant mass (12–14d, 60°C), and weighed.

We compared families of progeny produced from hand cross-fertilization vs. hand self-fertilization for all three style morphs in percent emergence, total number of flowers, and total biomass. Restricted maximum likelihood was used to test for significance of differences (PROC MIXED; Littell et al., 1996; SAS Institute, 2002–2005). The degrees of freedom for the fixed effects were estimated using the Kenward-Roger correction which reduces the probability of making a Type I error for small samples sizes and unbalanced designs (Kenward and Roger, 1997; SAS Institute, 2002–2005). Treatment category (progeny produced via self-fertilization, or cross-fertilization), maternal style-morph category (short-, mid-, and long-styled) and interactions between treatment and maternal style-morph were considered fixed effects. Maternal family (nested within maternal morph) and interactions between treatment category and maternal family (nested within maternal morph) were considered random effects. We used residual likelihood values comparing the full model (with both random effects) to reduced models and tested against a χ^2 distribution with one

degree of freedom (Littell et al., 1996). We calculated relative fitness for each maternal family as (w_s/w_o), where w_s and w_o are the values for selfed and outcrossed progeny, respectively, for life stages including percent emergence, total number of flowers, and total biomass. Cumulative relative fitness for each family was calculated as the product of the relative fitness for percent emergence, total number of flowers and total biomass; these latter three traits were not significantly correlated with each other in any pairwise comparison ($P > 0.05$ for all three comparisons, $n = 23$ maternal families).

Because of the importance of SC of the mid-styled morph for models of the breakdown of tristylly (Charlesworth, 1979), we also used only the progeny of the 16 mid-styled families to determine whether SC families with a likely history of inbreeding showed reduced inbreeding depression (δ). Using these families, we compared percent emergence, number of flowers, and total biomass of progeny produced from hand cross-fertilization vs. hand self-fertilization. Statistical approaches were similar to those used for the analysis that included all three style morphs. We investigated whether differences in likely inbreeding history of mid-styled families are associated with reduced inbreeding depression. We calculated relative fitness (w_s/w_o) for each of 16 mid-styled maternal families for life stages including percent emergence, average number of flowers per inflorescence, total number of flowers, total number of inflorescences, and total biomass. Cumulative relative fitness for each family was calculated as the product of the relative fitness for percent emergence, total number of flowers, and total biomass. We used two-tailed Mann-Whitney U tests to examine if the relative fitness of selfed progeny from highly self-compatible mid-styled individuals ($n = 5$) was different than that of selfed progeny from less self-compatible mid-styled individuals ($n = 11$) for the traits of percent emergence, number of flowers, total biomass, and cumulative relative fitness. The relative fitnesses of selfed progeny from autogamous mid-styled individuals ($n = 7$) vs. selfed progeny of nonautogamous mid-styled individuals ($n = 9$) at multiple life stages were also compared using two-tailed Mann-Whitney U tests. We examined correlations between the convergence index (stigma-anther separation) and family-level relative fitness (percent emergence, number of flower, total biomass, and cumulative relative fitness) for mid-styled families using Spearman rank correlations.

RESULTS

Frequency of autogamy for short-, mid-, and long-styled individuals—The frequency of individuals that produced capsules autogamously varied by style morph. Autogamous capsules were produced by 17% (4/24) of short-styled individuals, 44% (7/16) of mid-styled individuals, and no (0/30) long-styled individuals.

Self-compatibility and autogamy—Following artificial crosses, seed production per pollination did not differ among floral morphs ($F_{2,56} = 1.09$; $P = 0.3426$) and there was no significant interaction between floral morph and pollination treatment ($F_{3,56} = 1.69$; $P = 0.1805$). Pollination treatment had a significant effect on mean seed production ($F_{2,56} = 101.53$; $P < 0.0001$). Cross-fertilized flowers (19.07 ± 1.09 seeds per pollination, $n = 26$ maternal plants) had greater seed production than all other floral treatments (Fig. 3). Self-pollinated capsules (2.47 ± 0.55 seeds per pollination, $n = 26$ maternal plants) and autogamously produced capsules (2.99 ± 0.48 seeds per capsule, $n = 11$ maternal plants) had similar seed production. In mid-styled individuals, high SC individuals had greater autogamous seed production per capsule than low SC individuals ($z = 3.25$; $n_1 = 11$, $n_2 = 5$; $P = 0.0012$; Fig. 4). Seed production from flowers that were tagged but not pollinated by hand was significantly different from zero only in mid-styled individuals ($t = 2.56$; $df = 14$; $P = 0.0216$).

Stigma-anther convergence—The convergence index of mid-styled individuals did not change between early and later flowering periods ($t = 1.54$; $df = 10$; $P = 0.1551$). Among

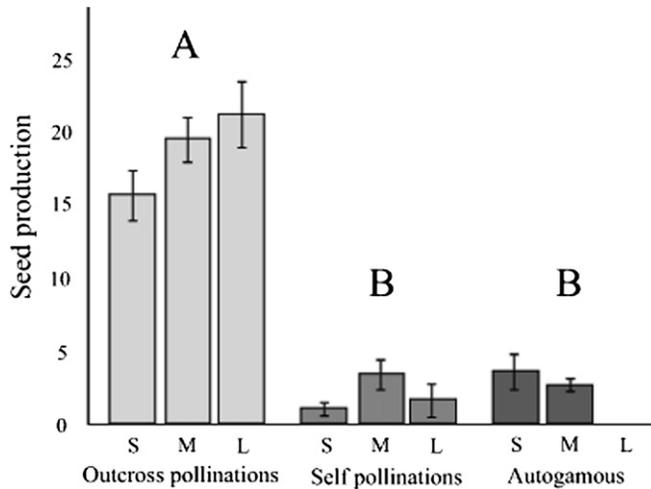


Fig. 3. Mean seeds produced (+/- one standard error) in three different treatment categories (outcross, self, autogamous) by style morph (S, M, L). Seed production for outcross- and self-pollinations was calculated as mean seeds produced per pollination, seed production for the autogamous treatment was calculated as mean seeds produced per pollination. Letters above bars indicate treatment categories that differed significantly from other treatments categories (when style morphs are pooled) in Tukey posthoc tests.

mid-styled plants, autogamous individuals had less stigma-anther separation than nonautogamous individuals ($z = 2.70$; $n_1 = 9$, $n_2 = 7$; $P = 0.0069$; Fig. 4). High SC and low SC mid-styled individuals did not differ in stigma-anther separation ($z = 1.74$; $n_1 = 11$, $n_2 = 5$; $P = 0.0810$).

Inbreeding depression: Population level—Maternal families of short-, mid-, and long-styled individuals (23 maternal families) responded differently to cross- and self-fertilization at all life history stages measured (percent emergence, total number of flowers, total number of inflorescences, average number of flowers per inflorescence, and total biomass), as shown by significant interactions between treatment category (progeny produced via self-fertilization or cross-fertilization) and maternal

family nested within maternal style morph (short-, mid-, or long-styled; Table 1). The effect of cross- and self-fertilization on percent emergence depended upon style morph as shown by a significant interaction ($F_{2,37.3} = 3.60$; $P = 0.04$). Style morphs differed in total number of flowers (short-styled = 21.06 ± 3.54 ; mid-styled = 18.80 ± 1.41 ; long-styled = 46.83 ± 3.11 ; $F_{2,30.1} = 7.25$; $P = 0.003$) and total number of inflorescences (short-styled = 4.62 ± 0.68 ; mid-styled = 4.05 ± 0.27 ; long-styled = 9.96 ± 0.51 ; $F_{2,30.1} = 7.49$; $P = 0.002$). Biomass showed significant inbreeding depression at the population level ($\delta = 0.35$; $F_{1,40.3} = 16.14$; $P = 0.0002$; Table 1). For the population, cumulative relative fitness was 0.59 ± 0.20 , weighted evenly by all three floral morphs ($n = 23$ families).

Inbreeding depression in mid-styled families: Family-level relative fitness—For the 16 mid-styled maternal families, the effect of pollination treatment (cross- vs. self-pollination) varied among maternal families for all life stages except biomass (significant family by life stage interactions, Table 2; Fig. 5). Total flower number, total inflorescence number, and biomass showed significant inbreeding depression. Cumulative relative fitness was 0.44 ± 0.11 ($n = 16$ mid-styled families).

In mid-styled individuals, selfed progeny of high SC individuals had higher cumulative relative fitness (RF = 0.81) than low SC individuals (RF = 0.28; $z = 2.04$; $n_1 = 11$, $n_2 = 5$; $P = 0.0414$; Fig. 4, 5). Selfed progeny from autogamous mid-styled individuals and selfed progeny from nonautogamous mid-styled individuals had similar values for cumulative relative fitness. The convergence of stigmas and anthers was not correlated with relative fitness at any life cycle stage in mid-styled families.

DISCUSSION

We demonstrate that within a largely self-incompatible tristylous population, maternal families with a history of inbreeding (inferred from increased SC) have lower levels of inbreeding depression (higher relative fitness of selfed to outcrossed progeny), suggesting that purging of inbreeding depression has occurred. In the Sierra La Mariquita population of *Oxalis alpina*, this pattern of purging was most evident in highly self-compatible

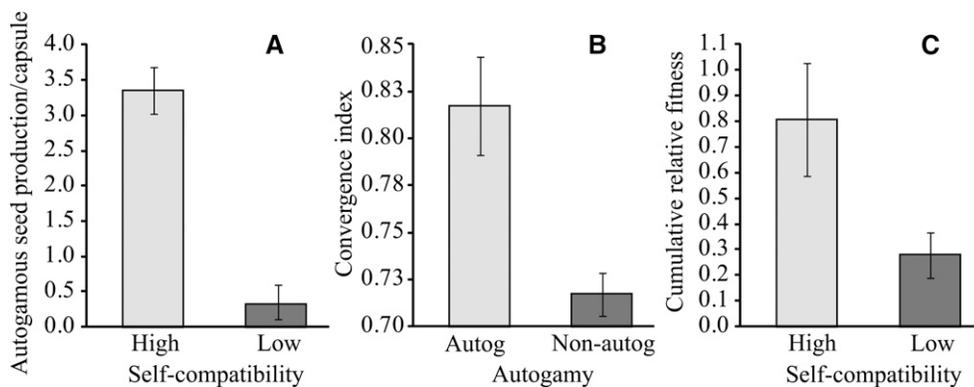


Fig. 4. A. Autogamous seed production per capsule for mid-styled individuals with high and low self-compatibility (two-tailed test; $z = 3.25$; $n_1 = 11$, $n_2 = 5$; $P = 0.0012$). Individuals with high self-compatibility produce more seeds per capsule than individuals with low self-compatibility. B. Convergence index of autogamous (Autog) and nonautogamous (Nonautog) mid-styled individuals (two-tailed test; $z = 2.70$; $n_1 = 9$, $n_2 = 7$; $P = 0.0069$). As the convergence index approaches one, long-level anthers and mid-level stigmas are more similar in length. Autogamous mid-styled individuals exhibited greater convergence. C. Cumulative relative fitness (w_s/w_o), of mid-styled families with high and low self-compatibility (two-tailed test; $z = 2.04$; $n_1 = 11$, $n_2 = 5$; $P = 0.0414$). Mid-styled families with greater self-compatibility exhibit greater cumulative relative fitness. Error bars represent +/- one standard error.

TABLE 1. For 23 maternal plants (nested within style morph), fitness of progeny produced from hand self- and cross-fertilized capsules (treatment); df, F , P , and χ^2 are reported from restricted maximum likelihood analyses in PROC MIXED. We used residual likelihood values comparing a full model (with both random effects) to reduced models and tested against a χ^2 distribution with one degree of freedom.

Life-cycle stage	Source of Variation	Fixed effects			Random effects		
		df	F	P	df	χ^2	P
Percent emergence ¹	Treatment	1,39.5	2.55	0.12			
	Style morph	2,36.5	1.45	0.25			
	Treatment*Style morph	2,37.3	3.60	0.04			
	Family (Style morph)				1	4.30	0.038
	Treatment* Family (Style morph)				1	16.70	<0.0001
N flowers ²	Treatment	1,36.3	0.73	0.40			
	Style morph	2,30.1	7.25	0.003			
	Treatment*Style morph	2,33.5	0.95	0.40			
	Family (Style morph)				1	8.20	0.004
	Treatment* Family (Style morph)				1	30.10	<0.0001
N inflorescences ²	Treatment	1,38.9	0.62	0.44			
	Style morph	2,30.1	7.49	0.002			
	Treatment*Style morph	2,35.7	0.88	0.42			
	Family (Style morph)				1	10.00	0.002
	Treatment* Family (Style morph)				1	24.50	<0.0001
N flowers/ inflorescence	Treatment	1,22.6	0.59	0.45			
	Style morph	2,24.4	0.53	0.60			
	Treatment*Style morph	2,21.8	0.40	0.68			
	Family (Style morph)				1	7.90	0.005
	Treatment* Family (Style morph)				1	21.10	<0.0001
Biomass (mg)	Treatment	1,40.3	16.14	0.002			
	Style morph	2,33.7	1.36	0.27			
	Treatment*Style morph	2,34.2	0.35	0.71			
	Family (Style morph)				1	11.40	<0.0007
	Treatment* Family (Style morph)				1	2.70	0.100

¹Percent emergence was arcsine, square-root transformed.

²Dependent variables were square-root transformed.

mid-styled families. Although predicted by theory (Uyenoyama and Waller, 1991a, b, c), a relationship between inbreeding history and inbreeding depression for families within a population has been shown in only a few empirical studies (Daehler, 1999;

Vogler et al., 1999; Takebayashi and Delph, 2000; Stone and Motten, 2002; Escobar et al., 2009). High levels of variation among individuals in levels of self-fertilization and inbreeding depression may be prerequisites for these associations to develop

TABLE 2. For 16 mid-styled maternal plants, fitness of progeny produced from hand self- and cross-fertilized capsules (treatment); df, F , P , and χ^2 are reported from restricted maximum likelihood analyses in PROC MIXED. We used residual likelihood values comparing a full model (with both random effects) to reduced models and tested against a χ^2 distribution with one degree of freedom.

Life-cycle stage	Source of Variation	Fixed effects			Random effects		
		df	F	P	df	χ^2	P
Percent emergence ¹	Treatment	1,12.0	0.21	0.66			
	Family				1	1.90	0.168
	Treatment* Family				1	18.20	<0.0001
N flowers ²	Treatment	1,16.1	7.57	0.01			
	Family				1	3.80	0.051
	Treatment* Family				1	31.90	<0.0001
N inflorescences ²	Treatment	1,16.1	6.60	0.02			
	Family				1	4.90	0.027
	Treatment* Family				1	28.50	<0.0001
N of flowers/ inflorescence	Treatment	1,12.3	4.23	0.06			
	Family				1	7.60	0.006
	Treatment* Family				1	11.80	<0.0001
Biomass (mg)	Treatment	1,14.9	36.43	0.001			
	Family				1	13.60	<0.0001
	Treatment* Family				1	1.90	0.168

¹Percent emergence was arcsine, square-root transformed.

²Dependent variables were square-root transformed.

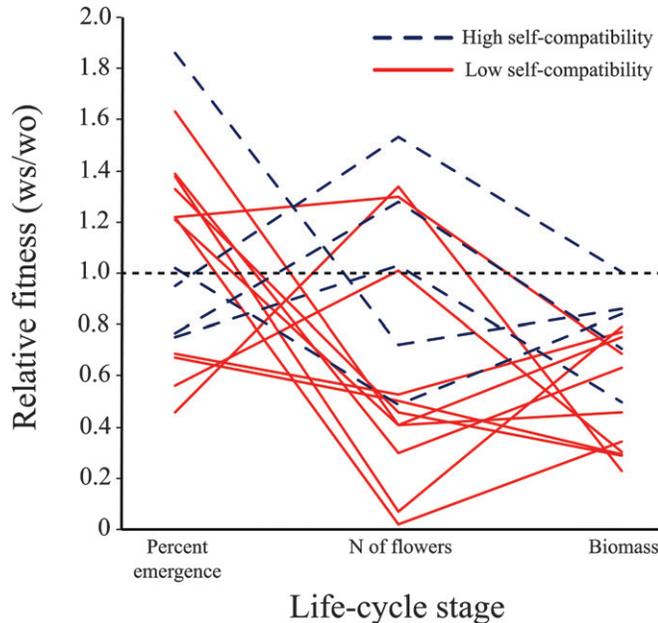


Fig. 5. Relative fitness (w_s/w_o) for 16 mid-styled families at three life stages. Relative fitness varied significantly among maternal families for all life stages except biomass. Maternal families from plants with high self-compatibility (dashed lines; $n = 5$) exhibited higher relative fitness than maternal families from plants with low self-compatibility (solid lines; $n = 11$).

between these traits (Takebayashi and Delph, 2000; Uyenoyama and Waller, 1991a, b, c). The greater propensity for SC and autogamous capsule production in mid-styled plants (relative to short- or long-styled plants) of this tristylous species appears to have facilitated the potential for genetic associations to develop between inbreeding history and inbreeding depression.

Evidence for lineage-specific inbreeding depression within the mid-styled morph—Among mid-styled individuals, progeny of highly self-compatible individuals had reduced inbreeding depression compared to progeny of individuals with low SC, suggesting that purging of deleterious alleles has occurred in these families. Self-compatibility probably evolved before autogamy, because autogamy is possible only in self-compatible individuals (Busch and Schoen, 2008). The absence of detectable associations between stigma-anther separation and relative fitness may imply that reduced stigma-anther separation evolves only after the evolution of associations of self-compatibility with relative fitness, and that these associations have appeared recently. Overall, our results are consistent with the theory that lineage-specific inbreeding depression can co-evolve with genetically controlled mating system modifiers that influence SC (Uyenoyama et al., 1993).

Our results add to a small body of empirical work demonstrating associations between inbreeding history and inbreeding depression at the family level. In fully self-compatible angiosperms, studies in two species found an association of reduced inbreeding depression with reduced stigma-anther separation, a heritable trait used as an indicator of self-fertilization for families of *Gilia achilleifolia* Benth. (Polemoniaceae; Takebayashi and Delph, 2000; Takebayashi et al., 2005) and *Datura stramonium* L. (Solanaceae; Stone and Motten, 2002). Similar associations between inbreeding depression and mating system traits

have been demonstrated in taxa with variation in SC or levels of selfing within a population. In the hermaphroditic snail, *Physa acuta* Draparnaud (Physidae), individuals exhibited variation in the time before self-fertilization occurred, a life-history trait that influences the mating system (Tsitroni et al., 2003). Individuals with greater potential to produce offspring with strong inbreeding depression delayed self-fertilization relative to individuals with less potential to produce offspring exhibiting inbreeding depression (Escobar et al., 2009). In an invasive species of cordgrass, *Spartina alterniflora* Loisel. (Poaceae), the degree of self-fertility (based on the proportion of ovules self-fertilized, ranging from 8–72%) and level of inbreeding depression was negatively correlated in competitive and low nutrient environments (Daehler, 1999). Family-level inbreeding depression and degree of self-incompatibility was positively correlated in *Campanula rapunculoides* L. (Campanulaceae), where individuals derived from strongly self-incompatible plants exhibited more severe inbreeding depression than individuals derived from self-compatible plants (Vogler et al., 1999).

In other studies, inbreeding history was not correlated with significant variation in inbreeding depression among families (Carr et al., 1997; Mutikainen and Delph, 1998; Chang and Rausher, 1999). Significant variation among families in inbreeding depression may indicate random differences in genetic load among families, and not necessarily lead to associations between inbreeding history and inbreeding depression (Schultz and Willis, 1995). Chang and Rausher (1999) found that mating system alleles were not strongly associated with alleles at inbreeding depression loci in *Ipomoea purpurea* (L.) Roth (Convolvulaceae), where individuals with greater stigma-anther separation exhibited only a nonsignificant trend toward greater inbreeding depression. Levels of self-fertilization as well as inbreeding depression must be highly variable among individuals within a population for associations to develop (Takebayashi and Delph, 2000; Uyenoyama and Waller, 1991a, b, c). Additionally, for discernable associations to develop between inbreeding depression and inbreeding history, models predict that selfing levels should fall within a range that allows genetic associations to develop but precludes fixation of the selfing allele (Schultz and Willis, 1995). Pollen flow studies in the Sierra La Mariquita population (F. Baena-Díaz and C.A. Dominguez, personal communication, Universidad Nacional Autónoma de México) indicate that the mid-styled morph is more highly self-pollinated than the short- and long-styled morphs, although we were unable to quantify natural levels of self-fertilization for the maternal parents used in our experimental families. Measures of levels of self-fertilization and inbreeding depression under field conditions would provide a more complete test of these models.

Variation among families in levels of self-fertilization and inbreeding depression may be quite pronounced in breeding systems with different reproductive morphs, such as heterostyly (Barrett et al., 1989). Among floral morphs in the Sierra La Mariquita population, mid-styled individuals exhibited greater SC and ability to produce fruits autogamously than short- or long-styled individuals. These traits were highly variable among mid-styled families, indicating that the level of self-fertilization among mid-styled families may also be highly variable. Inbreeding depression at the family level was also quite variable in this population. If levels of self-fertilization in families vary through time, it seems unlikely that associations with inbreeding history and inbreeding depression within a population will develop. Alternatively, these associations may elude detection

if selfing alleles move rapidly to fixation in a population. The ability to detect relationships between SC and inbreeding depression in mid-styled morphs of *Oxalis alpina* suggests that both of these genetically-based traits are important under field conditions.

Differences in inbreeding depression among floral morphs—Greater purging of inbreeding depression (i.e., higher relative fitness of selfed progeny) was expected in mid-styled families because this floral morph typically has greater SC than short- and long-styled morphs throughout populations of *Oxalis alpina* (Weller et al., 2007). In contrast to these expectations, the cumulative relative fitness of selfed progeny from mid-styled families (RF = 0.44 ± 0.11) was 24% lower than the relative fitness of selfed progeny exhibited by the population as a whole. The cumulative relative fitness of selfed progeny from mid-styled families was lower by 33% when compared to only short- and long-styled families (RF of short- and long-styled families = 0.66 ± 0.33). While some mid-styled families are SC and show evidence of purging, other mid-styled families are very self-incompatible and exhibit low fitness of selfed progeny. Among SC families, those that have recently evolved SC are likely to exhibit lower relative fitness of progeny derived from selfing compared to families that may have self-fertilized for many generations; these factors might explain the occurrence of lower relative fitness for mid-styled morphs as a group. Comparisons of inbreeding depression among families of different style-morphs should be interpreted with caution because the strong self-incompatibility of short- and long-styled morphs resulted in a smaller sample of families used to estimate inbreeding depression.

Family-specific inbreeding depression and implications for heterostyly—The development of lineage-specific inbreeding depression within a population could have implications for the evolution of heterostylous breeding systems, specifically the transition from tristylous to distylous. In *Oxalis alpina*, the mid-styled morph has been lost during the transition from tristylous to distylous in several populations (Weller, 1976a; Weller et al., 2007). Theoretical models (Charlesworth, 1979) predict that the evolution of SC may be critical in determining whether mid-styled individuals are lost, because SC would allow self-fertilization and expression of inbreeding depression, leading to eventual loss of the mid-styled morph from the population (Charlesworth, 1979). The evolution of self-fertilization has been described in multiple heterostylous taxa (Kohn et al., 1996; Schoen et al., 1997; Mast et al., 2006; Barrett et al., 2009); in many cases, this transition has been accompanied with morphological changes that may aid in self-fertilization (Stout, 1925; Ornduff, 1972; Barrett, 1988; Belaoussoff and Shore, 1995). Progeny of self-fertilizing individuals with the same style morph may inherit mating system modifiers (e.g., reduced stigma-anther separation; increased self-compatibility) that promote selfing. In mid-styled lineages capable of autogamous self-fertilization and purging of deleterious mutations, the cost of inbreeding is reduced. Purging of inbreeding depression in mid-styled lineages may explain the low, but potentially stable frequencies of mid-styled individuals in populations of *Oxalis alpina* where genic selection would otherwise favor loss of the mid-styled morph (Weller et al., 2007).

Some short-styled individuals in the Sierra La Mariquita population also exhibited the ability to self-fertilize and produced seeds autogamously. Unfortunately, because only a few

short-styled plants were partially autogamous ($n = 4$) we could not examine associations between relative fitness of selfed progeny of short-styled families and traits such as SC that may influence the mating system. Some models suggest that recessive deleterious alleles may be linked to the *S-locus*, which is typically heterozygous (Mather and de Winton, 1941). As a consequence, these recessive deleterious alleles are not normally expressed and exposed to selection. If deleterious alleles are linked to the *S-locus*, the relative fitness in selfed progeny of short-styled plants could be lower than relative fitness in the progeny of the other morphs (Mather and de Winton, 1941; Barrett et al., 1989). In contrast to these predictions, we found high cumulative relative fitness of selfed progeny in short-styled families, although our sample size for short-styled families was quite small (RF = 0.99 ± 0.30 ; $n = 5$ maternal families). Other studies have also found that selfed progeny of short-styled families have similar relative fitness to selfed progeny of mid- and long-styled plants (*Pontederia cordata* L. (Pontederiaceae); Gettys and Wofford, 2008; *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae); Manicacci and Barrett, 1996). Though relative fitness of selfed progeny in short-styled families was very high in *Oxalis alpina*, the potential for short-styled self-fertilizing lineages to evolve is low because we have observed very little self-compatibility in short-styled plants across populations (Weller et al., 2007).

Conclusions—Our study demonstrates that family-specific inbreeding depression has evolved among families within a population; offspring of highly self-compatible individuals exhibited lower inbreeding depression than offspring of less self-compatible individuals. Few studies have demonstrated that variation in SC among families is positively associated with relative fitness of selfed offspring. In contrast to previous studies, we did not find that relative fitness is correlated with stigma-anther separation, suggesting that SC and autogamy may evolve before reduced stigma-anther separation. Associations within a population between mating system and fitness loci can impact the evolution and maintenance of self-fertilizing style morphs in heterostylous populations. We show that these associations can develop in a population with self-incompatibility, and influence the evolution of reproductive systems.

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