Inbreeding depression in two populations of *Arenaria uniflora* (Caryophyllaceae) with contrasting mating systems

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I used parallel family-structured crossing designs to investigate the relative performance of self and outcross progeny in selfing and predominantly outcrossing populations of the annual plant *Arenaria uniflora*. The selfer population experienced much lower inbreeding depression ($\delta = 0.05 \pm 0.02$ SE) than the outcrossers ($\delta = 0.19 \pm 0.02$ SE). The negative association between genetic load and selfing rate suggests that purgable partially recessive alleles are the primary source of inbreeding depression, as does its late expression in both populations. Inbreeding depression in the selfer population, which naturally consists of highly inbred lines, was used to calculate the mean dominance (h = 0.33) and incidence rate (U = 0.30) of deleterious mutations. In the outcrosser population, significant variation among individuals in the expression of inbreeding depression may reflect lineage-specific differences in inbreeding history or, more probably, random variation in mutational load. The low ($\ll 0.5$) inbreeding depression of outcrossers suggests that the maintenance of a mixed mating system in some *A. uniflora* populations and the evolution of nearly cleistogamous self-pollination in others may reflect local pollinator-mediated selection for selfing rather than the constant 3:2 genetic advantage invoked by many models.

Keywords: *Arenaria uniflora*, genetic load, inbreeding depression, mating system, selfing.

Introduction

Inbreeding depression is the major force opposing the evolution of self-fertilization in flowering plants and is a central element in most theories of mating system evolution (e.g. Lande & Schemske, 1985; Lloyd, 1992). The mating system and genetic load of a population interact. If the expression of deleterious partially recessive alleles is the primary source of inbreeding depression, the equilibrium genetic load will reflect the population's past history of inbreeding (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987, 1990; Charlesworth *et al.*, 1990b). High selfing rates facilitate efficient 'purging' of such alleles by exposing them to selection in the homozygous state, and feedback between reductions in genetic load and outcrossing rate will lead to the evolution of complete selfing once inbreeding depression drops below a threshold value set by selection for self-fertilization (Lande & Schemske, 1985; Charlesworth *et al.*, 1990b; Lande *et al.*, 1994). In

contrast, inbreeding depression due to nearly additive mildly deleterious alleles or overdominance at fitness loci can persist in inbred populations (Charlesworth & Charlesworth, 1990). However, in a population with a long history of inbreeding, variation at overdominant loci should be lost through drift or selection for the more fit homozygote, and inbreeding depression due to overdominance also reduced. Empirical studies of the relationship between inbreeding depression and selfing rate in natural populations generally find decreases in genetic load with increased selfing rate, consistent with the purging of deleterious alleles. (Carr & Dudash, 1996; Husband & Schemske, 1996; Johnston & Schoen, 1996). However, the relationship between inbreeding depression and selfing rate is often only weakly negative (Latta & Ritland, 1994), and many highly selfing populations maintain substantial levels of inbreeding depression (Holtsford & Ellstrand, 1990; Ågren & Schemske, 1993; Charlesworth *et al.*, 1994).

Variation in inbreeding depression among individuals, as well as the population mean, may be important in mating system evolution. The development of associations between loci affecting fitness and those controlling mating system can allow high selfing rates to evolve even

when deleterious recessives cause population mean inbreeding depression greater than selection for selfing (Holsinger, 1988; Uyenoyama & Waller, 1991). This results in the joint divergence of mating behaviour and genetic load among families, and has focused attention on individual levels of inbreeding depression rather than the population mean (Uyenoyama *et al.*, 1993). Depending on the genetic basis of inbreeding depression, such associations may also allow the maintenance of mixed mating.

Measures of inbreeding depression in natural populations provide a quantitative context for understanding ecological and genetic selection for self-fertilization. Here, I examine inbreeding depression in two populations of the annual plant *Arenaria uniflora* (Caryophyllaceae) with contrasting mating systems. These populations have been well characterized in previous studies of *A. uniflora* and represent extremes of selfing rate and floral morphology within the species (Wyatt, 1984, 1986; Fishman & Wyatt, 1999; Fishman, 2000). The evolution of autonomous selfing in *A. uniflora* appears be a case of reproductive character displacement resulting from local pollinator-mediated competition (Fishman & Wyatt, 1999). This provides a rare opportunity to examine inbreeding depression in a species in which the reproductive assurance value of selfing has been quantified.

In this paper, I present estimates of the timing and magnitude of reductions in inbred progeny performance in selfer and outcrosser *A. uniflora* and discuss factors affecting genetic load in the two populations. To further explore the genetic basis of inbreeding depression in *A. uniflora*, I calculate the dominance and mutation rate of deleterious alleles in the selfer population using methods developed for highly inbred lines (Charlesworth *et al.*, 1990a). I examine the distribution of inbreeding depression among families within each population and consider the role of within-population variation in genetic load in mating system evolution in *A. uniflora*. Finally, I compare the magnitude of inbreeding depression in these populations to the ecologically contingent benefits of self-fertilization.

Materials and methods

Study populations

Arenaria uniflora (Walt.) Muhl. (Caryophyllaceae) is a diploid ($2n = 14$) winter annual plant endemic to granite outcrops in the south-eastern United States. The species consists of large-flowered protandrous populations (outcrossers) in the centre of its range and small-flowered autonomously selfing plants (selfers) in marginal populations (Wyatt, 1984, 1986). The two *A. uniflora*

populations used in this study represent the extremes of mating system within the species. The outcrosser population (Pendergrass, GA; Wyatt's [1984] population 7), is highly protandrous and individual outcrossing rates measured in naturally pollinated arrays indicate a mixed mating system ($t = 0.77 \pm 0.09$ SE; Fishman, 2000). In contrast, plants from the selfer population (Liberty, SC; Wyatt's [1984] population 8) are nearly cleistogamous, generally self-pollinating in the bud. Low rates of pollen import from outcrossers ($< 10\%$) and export to outcrossers ($< 2\%$) indicate the likelihood of pollen transfer among selfers is vanishingly low (Fishman, 2000).

Despite their striking floral and mating system divergence, the Liberty, SC and Pendergrass, GA populations are geographically and genetically allied. The populations occur on small granite outcrops separated by ~ 100 km and both populations consist of large numbers of individuals with mean densities at flowering of at least 500 plants/m^2 (Wyatt, 1986). Allozyme and crossability data indicate that the Liberty selfer population is more closely related to the Pendergrass outcrossers than to more distant *A. uniflora* populations, including other selfers (Wyatt, 1990; Wyatt *et al.*, 1992). See Wyatt (1984, 1986) for detailed locations and descriptions of these populations.

Crossing design

Parallel family structured crossing designs were used to examine the fitness consequences of selfing in the two populations. This approach allows estimation of within-population variation in genetic load and the construction of confidence intervals around population mean inbreeding depression (Johnston & Schoen, 1994). Within each population, the performance of self and outcross half-sib families was measured across life stages from seed to reproductive adult.

Dormant rosettes of selfers and outcrossers were collected in early March 1996 and transplanted into 8 cm square plastic pots containing a thin layer of field soil over coarse sand. The plants were raised in an insect-free greenhouse at the University of Georgia, which was kept cool (15°C) and naturally lit throughout the growing season. Before flowering, plants from each population were randomly selected as recipients for self and outcross hand-pollinations ($N = 32$ selfers and 36 outcrossers). The crossing design was slightly different for the two populations because of the pseudocleistogamy of the Liberty selfer population.

Flowers of outcrossers were emasculated at anthesis and hand-pollinated 3–4 days later when the stigmatic lobes became receptive. In addition to outcross and self treatments for estimating inbreeding depression, the Pendergrass plants received five other pollination

treatments discussed elsewhere (Fishman & Wyatt, 1999). Outcrossed flowers received pollen from ≥2 donors per pollination from a separate pool of 100 donor plants. Selfed flowers received pollen from younger flowers on the maternal plant. About five flowers on each plant received each treatment over a 4-week period, with an effort made to pollinate each plant with both treatments on any given day. A lack of fruit set by emasculated control flowers indicated that there was little illicit pollen transfer in the insect-free greenhouse.

Because Liberty selfer plants routinely self-pollinate in the bud, it was necessary to emasculate selfer flowers several days prior to anthesis. To reduce work and wastage of damaged flowers, only outcross treatment flowers were emasculated. Under a dissecting microscope, the petals and anthers were removed with forceps and the stigma inspected for pollen. The stigma and ovary were inspected again after 2–3 days and, if free of pollen and undamaged, pollinated with outcross pollen. Outcross pollination of selfers consisted of mixed pollen from ≥ 2 donors from a separate pool of selfer plants. Self flowers were allowed to set seed autonomously because preliminary crosses had indicated that supplemental hand-pollination does not increase the seed set of selfers. Approximately four flowers on each plant received outcross pollen and six flowers were allowed to set seed autonomously. Because *A. uniflora* flowers generally close and begin to form fruits within six hours of pollination (Fishman, personal observation), it is unlikely that flowers receiving outcross pollen had autonomously self-pollinated.

Measurement of inbreeding depression

Mature fruiting capsules from all crosses were collected 2–3 weeks after pollination. At the end of the season, the seeds from each treatment × maternal plant combination were pooled, then counted and weighed in bulk. Seed set (seeds per flower) and mean seed mass (mg) were calculated. Germination of each seed pool was assayed in Parafilm-sealed Petri dishes containing moist sand. Seeds were after-ripened at 30°C/15°C (12 h/12 h cycle) in an environmental control chamber for three months, then cooled to 15°C/6°C to initiate germination. Total percentage germination was recorded after one month of cool temperatures.

Post-germination components of fitness were measured in a greenhouse at Princeton University. Although it was not possible to test progeny performance in the field, growing conditions were chosen to mimic natural conditions. As with the parental generation, plants were grown in a thin layer of field soil over coarse sand and watered from below until well-established. The greenhouse was kept at ~10°C for six weeks to ensure vernalization, then warmed to 15–20°C to initiate shoot production and flowering. Daylength was not manipulated, but mercury vapour lamps provided supplemental light during the day. The end of the flowering season was effected by allowing the plants to gradually dry out. Progeny arrays from the two populations were grown on adjacent benches, rather than intermixed, because differences in size between selfers and outcrosser plants would have resulted in asymmetric competition between the populations, a potentially greater confounding factor.

Some families from each population had complete germination failure in one or both pollination treatments and were excluded from postgermination tests of performance to balance the design. Thirty-two outcrosser families and 26 selfer families provided sufficient seedlings for inclusion in the progeny arrays. Progeny were grown in square trays consisting of 64 25 cm² cells, so that each replicate block contained a full complement of self and outcross families (32 dams × 2 cross types). For the selfers, families with excess seedlings were substituted for missing families, so that six maternal families (both self and outcross) were replicated within blocks. Families were assigned random positions within each array.

Because intraspecific competition or other stresses can increase inbreeding depression (e.g. Schemske, 1983; Dudash, 1990; Wolfe, 1993), I measured postgermination performance at two densities. Half of the total number of arrays (outcrosser $N = 10$; selfer $N = 6$) were planted at high density (six seedlings per cell) and half at low density (two seedlings per cell). Crowding in the low density arrays was comparable to late season field densities of both populations. Seedlings that died within two weeks of transplanting ($< 5\%$) were replaced with seedlings from the same cross. This mortality did not vary across populations or other variables and was probably due to damage during transplanting. Subsequent mortality was very low ($< 2\%$). Total progeny performance was measured for each cell and divided by the original planting density. Thus, mortality is included in the performance measures, as is compensatory growth by siblings within the cell.

I obtained three measures of adult performance: shoot production, early flowering and fecundity. Shoot number was counted when plants were still in the rosette stage, before shoot elongation and flowering. An early count of flowers was made eight weeks after transplanting, when some families had not yet begun to flower, and estimates the timing of flowering. Fecundity was measured at the end of the flowering season by counting all flowers and fruits. The persistent sepals and pedicels of *A. uniflora* allow counting of past flowers even when no fruits have formed. In the nearly cleistogamous

selfers, flower number is a good estimator of female fitness because most flowers have full fruit and seed set in the absence of pollinators (Fishman, 1998). Outcrosser fruit set is more dependent on the pollination environment, so flower production puts an upper bound on female fecundity.

Statistical analyses

Analyses were performed using the standard least squares analysis of variance platform in JMP 3.0.2 (SAS Institute, 1994). Progeny performance after selfing and outcrossing was analysed separately for the Liberty and Pendergrass populations. Maternal identity (dam) was included as a random effect in all ANOVA models. Cross type (selfed vs. outcrossed) was the main fixed effect. Analyses of particular response variables deviated from the general model as described below.

Seed performance variables were analysed with the general ANOVA model, which is equivalent to a paired *t*-test. Seed set and seed mass were analysed directly. Germination rates were arcsine–square root transformed before analysis to normalize the residuals. Because seeds from each dam × cross type combination were pooled for measurement, these analyses cannot identify between-family differences in response to selfing.

Adult performance variables were analysed with a more complex model, which included competition treatment and replicate blocks nested within competition treatment as well as family and cross effects. All two-way interaction terms involving cross were included in the final ANOVA model, but other two-way and three-way interactions that were nonsignificant ($P > 0.2$) in a preliminary full analysis were pooled with the error term. For *F*-tests, JMP 3.0.2 (SAS Institute, 1994) generates synthetic denominators based on interaction terms and the residual. For this model, all main effects other than cross were tested over the effect × cross interaction and all interactions were tested over the residual. Cross effects were tested over a synthetic denominator MS. The adult performance measures were *ln*-transformed and the data conformed to ANOVA assumptions after transformation. This transformation makes the significance of two-way interactions an appropriate test of differences between families or environments in inbreeding depression (Kalisz, 1989; Johnston & Schoen, 1994). In the outcrossers, a constant (0.25) was added to the shoot and flower values prior to transformation in order to include zero values. Overall inbreeding depression within each life stage was indicated by significant differences in performance between selfed and outcrossed progeny.

To examine the distribution of genetic load within and between populations, I calculated inbreeding depression ($\delta = 1 - w_s/w_o$, where w_s and w_o are the fitness of selfed and outcrossed progeny, respectively) for each maternal family. Because there was no evidence of inbreeding depression of seed characters, mean self and outcross fecundities (the back-transformed LSMs of the dam × cross type interaction from the ANOVA) were used to calculate lifetime inbreeding depression. However, analyses using a composite measure of fitness (seed set × percent germination × fecundity) produced similar mean values of inbreeding depression.

Population-level inbreeding depression was calculated as the mean of family δ-values. The mean of ratios does not necessarily equal the ratio of means, so this family structured estimate of inbreeding depression is not identical to the population-level measure used in most models of mating system evolution (Johnston & Schoen, 1994). However, the relatively large number of adult progeny measured in each dam × cross type family ($n_{selfers} = 6$ cells or 24 plants, $n_{outcrossers} = 10$ cells or 40 plants) assures that the mean of family level inbreeding depression in this study is close to the population mean. In simulations, Johnston & Schoen (1994) found that family sizes > 5 produce unbiased estimates of population-level inbreeding depression. The conventional metric of inbreeding depression ($\delta = 1 - w_s/w_o$) generates unbounded negative values when w_s exceeds w_o (i.e. when individual families show outbreeding depression), but does not bias the population mean at the range of relative fitnesses observed in this experiment. A symmetrical estimator of inbreeding and outbreeding depression (relative performance [RP]; Ågren & Schemske, 1993) produced values nearly identical to those reported.

Results

Fitness consequences of selfing across life stages

Neither population experienced inbreeding depression of seed performance (Fig. 1). Within the Pendergrass outcrossers, there were no significant differences between self and outcross pollinations in the number of seeds produced per fruit, mean seed mass or proportion germinated. The Liberty selfer population appeared to experience significant outbreeding depression of seed set (Fig. 1). This apparent outbreeding depression in the Liberty population probably resulted from the emasculation of only outcross treatment flowers, which sometimes damaged the recipient ovary. Visibly damaged flowers were not used in the experimental crosses, but some ovary damage may have gone undetected and reduced seed set in this treatment. The different prepollination treatment of selfed and outcrossed selfer flowers is unlikely to contribute to
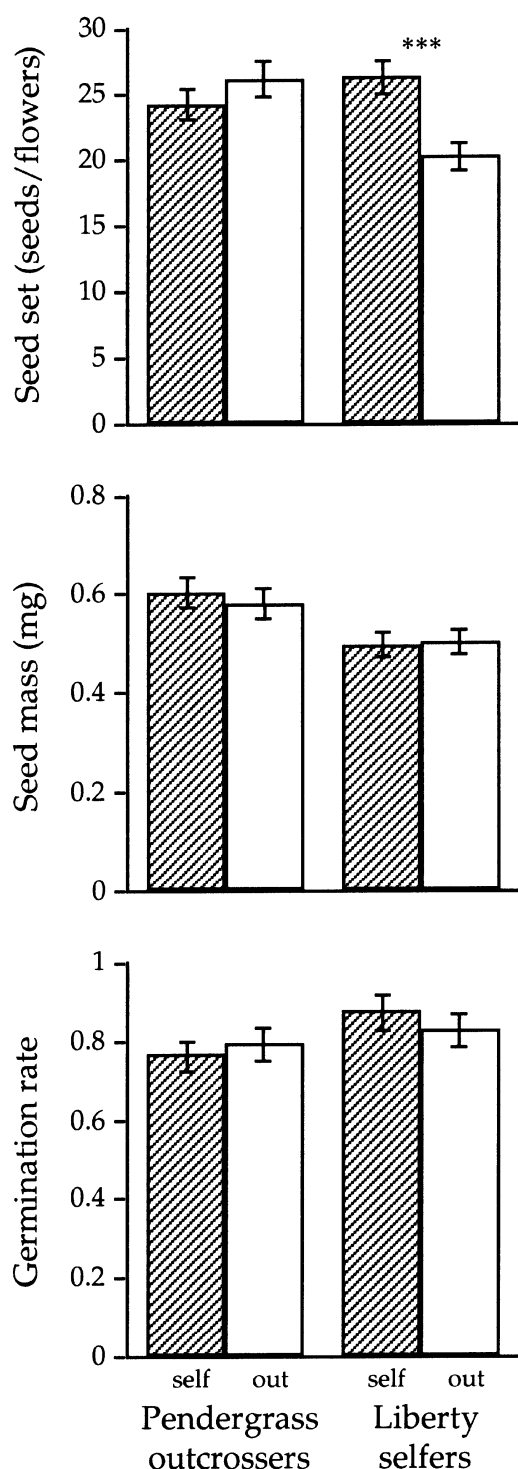
Fig. 1 Seed character means (± 1 SE) after self and outcross hand-pollinations of plants from outcrosser and selfer populations of *Arenaria uniflora*. Means are cross type effect LSMs (back-transformed if necessary) from separate analyses of variance for each population. Inbreeding depression (or, in the case of selfer seed set, apparent outbreeding depression) is indicated by significant differences between self and outcross performance (***$P < 0.001$).

patterns of fitness at later life stages because it did not significantly affect other measures of seed performance (Fig. 1) and the seed characters were never significant covariates in analyses of adult fecundity (data not shown).

Both populations exhibited strong family effects on components of seed performance across pollination treatments. The outcrossers showed highly significant maternal effects on both seed weight and percentage germination ($P < 0.0001$). A negative correlation between seed number and seed weight (Pearson correlation; $r = -0.37$, $P < 0.0001$) and a positive correlation between seed mass and germination rate ($r = 0.50$, $P < 0.0001$) suggest shared genetic or maternal effects. In the selfers, maternal identity strongly influenced seed set and seed mass ($P < 0.0001$), but did not affect germination rate. As in the outcrossers, seeds per fruit and seed mass were negatively correlated ($r = -0.32$, $P = 0.01$).

After seedling establishment, the magnitude of inbreeding depression in each population changed across the growing season (Fig. 2). In both selfers and outcrosser populations, outcrossed progeny produced ~20% more shoots than selfed progeny and cross type was a highly significant effect in the ANOVA. In the outcrossers, both shoot number and inbreeding depression of shoot production were dependent on maternal identity ($P < 0.01$). In the selfers, shoot number did not depend on maternal identity ($P = 0.21$), but levels of inbreeding depression of this character varied widely among families ($P < 0.001$).

Significant inbreeding depression of early flower production occurred in the selfers, but not in the outcrossers (Fig. 2). Families in both populations varied significantly in early flower production, but the effect of maternal identity was much stronger in the outcrossers ($P < 0.0001$ vs. $P = 0.018$), because many outcrosser plants were not yet flowering. In both populations, families differed substantially in inbreeding depression of early flowering ($P < 0.0001$).

At the end of the season, levels of inbreeding depression in the two populations were again quite different, with the outcrossers exhibiting greater inbreeding depression (Fig. 2; Table 1). The effect of cross was highly significant in the outcrossers ($F_{1;18.4} = 68$, $P < 0.0001$), with $\delta = 0.19 \pm 0.02$ SE calculated across families. Cross type also significantly affected fecundity in the selfers ($F_{1;5.6} = 8.4$, $P = 0.03$) but overall inbreeding depression was lower ($\delta = 0.05 \pm 0.02$ SE calculated across families). The fecundity of individuals in both populations was highly correlated with early shoot number (Pearson correlation of LSMs; $r_{selfers} = 0.72$ and $r_{outcrossers} = 0.68$). This suggests that differences in the initial production of
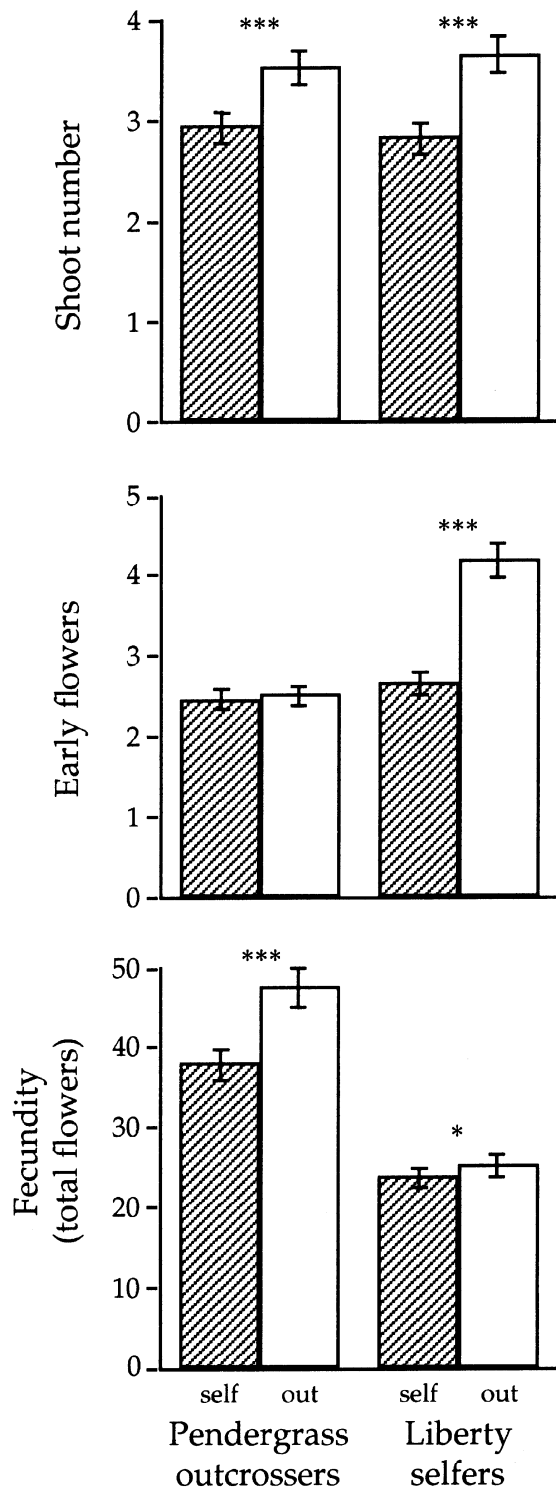
Fig. 2 Adult performance means (± 1 SE) of self and outcross progeny from outcrosser and selfer populations of *Arenaria uniflora*. Means are back-transformed cross type effect LSMs (which average across families, densities and arrays) from separate analyses of variance for each population. Inbreeding depression is indicated by significant differences between self and outcross performance (*** $P < 0.001$, * $P < 0.05$).

flowering shoots may determine later patterns of reproduction. Maternal identity had an only marginally significant effect on fecundity across pollination treatments ($P = 0.022$ and 0.064, respectively, for selfers and outcrossers).

With the exception of early flowering and shoot production in the selfer population, density had a strongly significant effect on adult performance ($P < 0.005$). Replicate arrays also varied significantly in mean performance for all characters ($P < 0.005$). In contrast, neither competition treatment nor array affected the relative performance of self and outcross progeny at any life stage, as indicated by the consistent nonsignificance of cross × array and cross × density interaction effects in both populations ($P > 0.20$).

Correlation of relative self and outcross performance across adult stages

Although the magnitude of inbreeding depression changed substantially across the growing season, the ranking of families remained fairly constant. In selfers, family level inbreeding depression of total fecundity was positively correlated with relative performance at both the shoot production ($r = 0.63$; $P < 0.001$) and early flowering stages ($r = 0.43$, $P < 0.05$). The outcrossers showed similar across-family correlations between inbreeding depression at the end of the season and at earlier stages. Although no population-level inbreeding depression of early flowering was detected in the outcrossers, variation among families in δ of fecundity was correlated with relative performance at early flowering ($r = 0.51$, $P < 0.005$).

Distribution of inbreeding depression among families

Selfers and outcrossers displayed different amounts of among-family variation in inbreeding depression of fecundity (Table 1, Fig. 3). In the Pendergrass outcrossers, a highly significant dam × cross type interaction reflects consistent differences in inbreeding depression among families ($F_{31;558} = 2.7871$; $P < 0.001$). In contrast, selfer families did not significantly differ from one another in the relative fecundity of selfed and outcrossed progeny ($F_{25,318} = 1.15$, $P > 0.25$). In the Pendergrass outcrossers, more than two-thirds of the families exhibited significant inbreeding depression. In the selfer population, only six of 26 families experienced significant inbreeding depression and eight families exhibited nonsignificant outbreeding depression (negative δ-values). Although family mean values of δ cover a wide range in both populations, variation in performance within selfer families overshadows the among-family variation.

Population	Source	d.f.	MS	F	P
Outcrosser (Pendergrass, GA)	Cross type	1	8.11	68.41	0.000
	Dam	31	0.25	1.74	0.064
	Density	1	171.47	926.38	0.000
	Array[density]	8	0.19	7.25	0.006
	Cross × dam	31	0.15	2.79	0.000
	Cross × density	1	0.03	0.83	0.388
	Cross × array[density]	8	0.03	0.49	0.863
	Error	558	0.05		
Selfer (Liberty, SC)	Cross type	1	0.31	8.42	0.029
	Dam	25	0.10	2.28	0.022
	Density	1	55.21	33.19	0.005
	Array[density]	4	1.66	54.78	0.001
	Cross × dam	25	0.04	1.15	0.285
	Cross × density	1	0.00	0.02	0.893
	Cross × array[density]	4	0.03	0.80	0.526
	Error	318	0.04		

Table 1 Nested mixed-model analyses of variance for the fecundity of self and outcross progeny of *Arenaria uniflora*. The outcrosser and selfer populations were analysed separately with parallel experimental designs and ANOVA models. Cross type (self vs. outcross) is a fixed effect and dam is a random maternal family effect. Array is a random block effect nested within the fixed effect of planting density. Fecundity values (total fruit and/or flower production) were *ln*-transformed for analysis. The same ANOVA model was used to analyse the other measures of adult performance
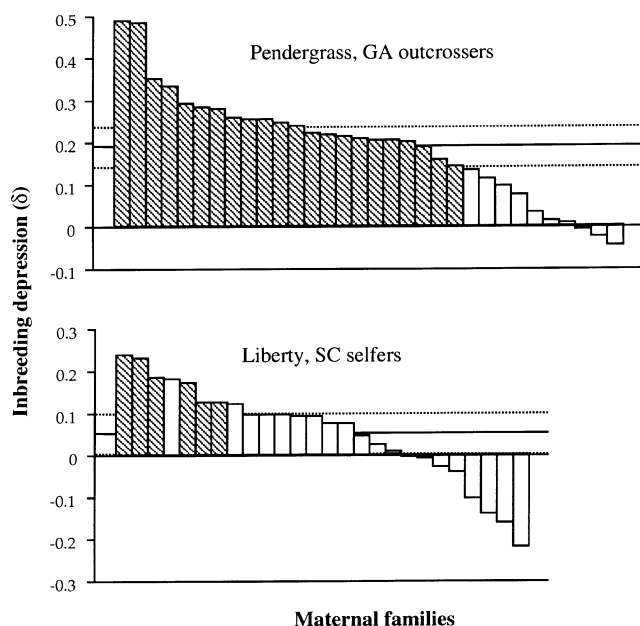

Fig. 3 Distribution of inbreeding depression of fecundity (δ) in the outcrosser and selfer populations of *Arenaria unoflora*. Each bar is the mean δ for a single maternal family ($N = 32$ and 26 dams for outcrossers and selfers, respectively). The hatched bars indicate families with inbreeding depression significantly different from zero in the ANOVA. The horizontal lines show the mean (solid) and 95% confidence interval (dotted) of inbreeding depression for each population as a whole.

Population-level effects of inbreeding

On average, plants from outcrosser and selfer populations experienced different levels of inbreeding depression of fitness. Inbreeding depression of total fecundity was much higher in the Pendergrass outcrosser population (mean $\delta = 0.19 \pm 0.02$ SE) than in the Liberty selfers (mean $\delta = 0.05 \pm 0.02$ SE). Estimates of population-level inbreeding depression made without explicitly incorporating family structure were 0.22 and 0.06, respectively, for outcrossers and selfers.

Discussion

The magnitude and timing of inbreeding depression

The lack of inbreeding depression of seed performance in both *A. uniflora* populations is consistent with the purging of early acting lethal recessive alleles. Even moderate levels of inbreeding (through selfing or during recent population bottlenecks) should be sufficient to remove such alleles from a population (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987). Other empirical studies have also confirmed this prediction (Husband & Schemske, 1996).

Lower inbreeding depression of fecundity in the obligate selfers relative to the outcrossers also supports the theory that genetic load due to deleterious recessives evolves jointly with selfing rate (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987, 1990; Charlesworth *et al.*, 1990b). However, in a population with a long history of routine inbreeding, fixation of the higher-fitness allele (given any asymmetry of homozygote fitness) would also eliminate genetic load due to overdominance at fitness loci Charlesworth & Charlesworth, 1990). In addition, drift may fix deleterious alleles in highly selfing populations. Without actually reducing the genetic load, such fixation could reduce

measured inbreeding depression (the lower fitness of selfed *relative* to outcrossed individuals), because all plants carry the same alleles. Very low inbreeding depression in highly selfing populations (e.g. this study, Barrett & Charlesworth, 1991; Parker *et al.*, 1995; Husband & Schemske, 1996; Johnston & Schoen, 1996) is probably due to a combination of these processes.

Both *A. uniflora* populations exhibit total inbreeding depression at the low end of the spectra for their observed mating systems. The values found in this study are much lower than the mean cumulative inbreeding depression values for selfing ($\delta = 0.22$) and outcrossing ($\delta = 0.49$) flowering plants reviewed by Husband & Schemske (1996). The moderate late-acting inbreeding depression in the Pendergrass outcrosser population (selfing rate < 0.3, $\delta = 0.19$) resembles that of a typical selfing species. The Liberty selfers, which have a selfing rate close to 1.0, experience negligible inbreeding depression of total fecundity ($\delta = 0.05$). Similarly low inbreeding depression has been reported for other highly selfing populations, including Jamaican *Eichornia paniculata* (Barrett & Charlesworth, 1991) and two species of *Amsinckia* (Johnston & Schoen, 1996). Other studies have found substantial inbreeding depression even in highly selfing taxa (Holtsford & Ellstrand, 1990; Karoly, 1991; Lyons & Antonovics, 1991; Ågren & Schemske, 1993). Low (< 0.20) levels of inbreeding depression in intermediately outcrossing species are less frequently reported, although some mixed-mating *Collinsia* populations appear similar to the *A. uniflora* outcrossers (Mayer *et al.*, 1996).

A number of factors (other than mating system *per se*) which reduce effective population size may contribute to the relatively low levels of inbreeding depression observed in these *Arenaria uniflora* populations. Both morphs of *A. uniflora* are restricted to isolated and highly seasonal granite outcrops, and drastic fluctuations in population size may have been a significant part of their population genetic histories. Biparental inbreeding and selfing after colonization events or other extreme demographic bottlenecks could have substantially reduced the genetic load of the currently mixed mating Pendergrass population (Lande & Schemske, 1985). Although estimates of the population outcrossing rate based on adult heterozygosity at a single locus suggest that the Pendergrass population is not highly inbred (Fishman, 1998), such estimates must be treated with caution because diversity at allozyme loci is low in all *A. uniflora* populations (Wyatt *et al.*, 1992). Measures of inbreeding depression in additional selfer and outcrosser populations of *A. uniflora* would help differentiate the roles of demographic history and mating system in influencing inbreeding depression, although these can never be entirely separated in natural populations.

Low levels of inbreeding depression could also result if the genetic load was not fully expressed under these experimental conditions. Perhaps inbreeding depression would have been higher under more stressful field conditions, which can amplify fitness asymmetries. Some comparative studies have found increased inbreeding depression in the field (Schemske, 1983; Dudash, 1990) or under competitive regimes (Schmitt & Ehrhardt, 1990; Wolfe, 1993), whereas others have detected no difference in self and outcross relative performance between environments (Charlesworth *et al.*, 1994; Nason & Ellstrand, 1995). In this study, higher density (3×) clearly provided a stressful environment, dramatically reducing fitness in both populations (outcrossers $F_{1;8} = 926.4$, $P < 0.001$; selfers $F_{1,4} = 33.2$, $P < 0.001$). However, increased competition had no detectable effect on the expression of inbreeding depression in either population. Although measuring total performance for each cell rather than for single individuals could have allowed vigorous self progeny to partially compensate for cellmates homozygous for deleterious alleles, such compensation would also have reduced the overall effect of competition on fitness. Because the strong, highly significant environmental effects on performance (density and array) did not affect the expression of genetic load, it is also highly unlikely that the observed between-population difference in inbreeding depression results from any environmental difference between the benches.

Estimation of dominance and mutation rate from inbreeding depression in selfers

The extremely high selfing rate of the Liberty selfer population provides the opportunity to make additional inferences about the genetic load in this species. If one assumes multiplicative effects of fitness loci and mutation–selection balance, inbreeding depression due to deleterious alleles in a completely self-fertilizing population is determined only by the dominance coefficient (h) and the mutation rate (Charlesworth *et al.*, 1990a). The harmonic mean degree of dominance can be calculated as the coefficient of the regression of outcross fitness (w_{ij}) on self fitness (w_{ii}), where *i* indicates a maternal parent and *j* is a sire chosen from the population at random, a modification the method of Johnston & Schoen (1995). Because maternal effects may also contribute to the regression of outcross on self sibling (parental) fitness, some caution must be used in interpreting estimated dominance coefficients. In this case, w_{ij} and w_{ii} were the cross × dam effect LSMs from the ANOVA of fecundity. In *Arenaria*, the regression of outcross on self sibling performance indicates partial recessivity of deleterious mutations (h = 0.33 ± 0.20 SE)

affecting fecundity in the selfer population. With only 26 families, this dominance coefficient cannot be statistically discriminated from either complete additivity (h = 0.5) or complete recessivity (h = 0) of deleterious alleles. However, the value for *A. uniflora* is quite similar to estimates of dominance coefficients from selfer populations of *Amsinckia* (Johnston & Schoen, 1995) and inbred lines of *Drosophila* (Charlesworth & Charlesworth, 1987).

The mutation rate (U) is approximated by U = [−2ln(1 − δ)]/1 − 2h (Charlesworth et al., 1990a). With a dominance estimate of 0.33 and mean inbreeding depression of fecundity of 0.05, the estimated per genome mutation rate in *A. uniflora* selfers is 0.30. This mutation rate is similar to those calculated for loci affecting overall fitness in two highly selfing populations of *Amsinckia spectabilis* (Johnston & Schoen, 1995), but lower than estimates from two autogamous *Leavenworthia* populations with substantial levels of inbreeding depression (Charlesworth et al., 1994). Although they do not have great statistical power, these calculations of dominance and mutation rate are in accordance with the growing consensus that inbreeding depression in plants has a common source in partially recessive deleterious mutations (Charlesworth et al., 1990a).

Variation in inbreeding depression among individuals

The two *A. uniflora* populations differ not only in their mean genetic load, but in the distribution of inbreeding depression among individuals. Maternal plants from the mixed mating Pendergrass population vary widely in the relative fitness of their self and outcross progeny (Table 1; Fig. 3). In contrast, Liberty selfer maternal plants do not significantly differ from one another in their levels of inbreeding depression of fecundity (Table 1, Fig. 3). Higher variability of inbreeding depression in outcrossers has also been reported for a pair of *Epilobium* species with contrasting mating systems (Parker et al., 1995). This pattern may simply arise from the long history of inbreeding in the selfer population, in which each lineage continuously purges all but the most mild (or least recessive) new deleterious mutations. Homogenization of the selfer population by recent genetic bottlenecks could also contribute to reductions in both inbreeding depression and variation in genetic load.

Variation among lineages in inbreeding depression, as observed in the outcrosser population, been suggested as a major factor in mating system evolution (Holsinger, 1988; Uyenoyama & Waller, 1991; Uyenoyama et al., 1993). In particular, stable mixed-mating systems can evolve when inbreeding depression is low and modifiers

that increase the outcrossing rate become associated with high viability genotypes (Uyenoyama et al., 1993). Differential purging, as well as such genetic associations, could create systematic variation in inbreeding depression within mixed mating populations. However, the individual variation in inbreeding depression observed in this and other mixed mating populations (e.g. Parker et al., 1995; Dudash et al., 1997) probably does not reflect individual inbreeding history, because it may not be possible to differentiate variation due to inbreeding history from random variation in mutational load. Random mutational variation can swamp variation due to inbreeding history, and variation at additive loci further prevents the detection of associations between individual genetic load and inbreeding depression (Schultz & Willis, 1995). Within-population variation in mutational load could provide an opportunity for the establishment of a selfing modifier that fortuitously occurs in a lineage with low inbreeding depression, but this does not appear to have happened in the mixed mating *A. uniflora* population. No relationship was observed between individual autogamy rate and inbreeding depression in *A. uniflora* outcrossers (Pearson correlation, r = 0.17, P = 0.63). Lineage-specific associations between mating behaviour and genetic load cannot be implicated in mating system evolution within this species.

Inbreeding depression relative to selection for self-fertilization

Inbreeding depression in the closely related Pendergrass population may be used to infer selection against self-pollination in outcrossing ancestors of the Liberty selfers. If selfing can be achieved without loss of outcross male fitness (i.e. if no 'pollen discounting' occurs: Holsinger, 1988), inbreeding depression must be greater than 0.5 to counteract the 3:2 advantage of selfer genes (Fisher, 1941; Jain, 1976). Thus, the population-level δ observed in the Pendergrass outcrossers (0.19 ± 0.02 SE) is much lower than the threshold value of 0.5 predicted by most models of the evolution of selfing (Lloyd, 1979; Lande & Schemske, 1985; Charlesworth et al., 1990b). Under the assumptions of such models, the evolution of autogamy in the Liberty population requires no further explanation and the maintenance of predominant outcrossing in the Pendergrass population is difficult to explain. However, additional processes appear to have been important in generating the current pattern of mating system variation in *A. uniflora*.

Localized pollinator-mediated selection appears to have favoured the evolution of autonomous self-pollination in *A. uniflora* (Fishman & Wyatt, 1999). Selfers

only occur in areas of sympatry with congener *A. glabra*, whereas outcrossers and *A. glabra* never co-occur (Wyatt, 1984, 1986). In field experiments, outcrossers suffered a 20–35% reduction in female reproductive fitness in the presence of *A. glabra* (Fishman & Wyatt, 1999). These losses primarily result from heterospecific pollen transfer by shared pollinators, ovule usurpation, and the production of inviable hybrid seeds. Even in the absence of an automatic transmission advantage, localized selection for pre-emptive self-pollination of this magnitude could have exceeded the moderate inbreeding depression observed in current outcrossers. Experimental measures of inbreeding depression and pollinator-mediated selection cannot fully reproduce ancestral conditions, but these results do suggest a quantitatively important role for reproductive assurance in mating system evolution.

Although the maintenance of predominant outcrossing in the Pendergrass population may simply reflect nonequilibrium conditions, it could also indicate constraints on mating system variation. If selfing variants experience trade-offs in male fitness, such that increases in self-pollination reduce pollen export, no constant selection for self-pollination occurs (Nagylaki, 1976; Holsinger, 1988) and selfing may not be favoured even when inbreeding depression is very low. In *A. uniflora*, the morphological and phenological changes associated with autonomous self-pollination prevent outcross pollen donation by the Liberty selfers, producing complete pollen discounting (Fishman, 2000). These trade-offs could be secondary adaptations to the selfing syndrome. However, quantitative genetic analysis suggest that pleiotropy contributes to associations between protandry and flower size in *A. uniflora* (Fishman, 1998). By imposing trade-offs between autogamous self-fertilization and pollinator attraction, such a genetic architecture could also constrain the male fitness of new selfing variants. Unfortunately, the magnitude and genetic basis of pollen discounting in initial selfing variants remains largely unknown, complicating predictions of the levels of inbreeding depression required to maintain outcrossing.

The joint evolution of inbreeding depression and selfing rate, suggested here for *Arenaria uniflora*, can be major factors in mating system evolution (Lande & Schemske, 1985; Charlesworth & Charlesworth, 1987). The low levels of inbreeding depression found in the autogamous *A. uniflora* population, as well as estimates of dominance and mutation rate, further implicate deleterious partially recessive alleles as the primary source of inbreeding depression in flowering plants. By allowing the application of simple genetic models to the evolution of self-fertilization, such a genetic basis could encourage the prediction of evolutionary trajectories

based only on measures of inbreeding depression. However, these results also emphasize that the magnitude of inbreeding depression alone cannot explain the evolution of selfing or the maintenance of outcrossing, and supports theoretical approaches that consider inbreeding depression in conjunction with empirical estimates of ecological and genetic selection for self-fertilization (e.g. Lloyd, 1979, 1992; Holsinger, 1992; Harder & Wilson, 1998).

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