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Increased inbreeding but not homozygosity in small populations of *Sabatia angularis* (Gentianaceae)

Rachel B. Spigler · J. L. Hamrick · Shu-Mei Chang

Abstract Understanding how the mating system varies with population size in plant populations is critical for understanding their genetic and demographic fates. We examined how the mating system, characterized by outcrossing rate, biparental inbreeding rate, and inbreeding coefficient, and genetic diversity varied with population size in natural populations of the biennial *Sabatia angularis*. We found a significant, positive relationship between outcrossing and population size. Selfing was as high as 40% in one small population but was only 7% in the largest population. Despite this pattern, observed heterozygosity did not vary with population size, and we suggest that selection against inbred individuals maintains observed heterozygosity in small populations. Consistent with this hypothesis, we found a trend of lower inbreeding coefficients in the maternal than progeny generation in all of the populations, and half of the populations exhibited significant excesses of adult heterozygosity. Moreover, genetic diversity was not related to population size and was similar across all populations examined. Our results suggest that the consequences of increased selfing for population fitness in *S. angularis*, a species that experiences significant inbreeding depression, will depend on the relative magnitude and consistency of inbreeding depression and the demographic cost of selection for outcrossed progeny in small populations.

Keywords Genetic diversity · Inbreeding coefficient · Heterozygosity · Mating system · Outcrossing rate · Population size

Introduction

The mating system of plant populations can impact population dynamics by influencing the average fitness of populations and their genetic composition in subsequent generations (Allard 1975; Frankel and Soulé 1981; Loveless and Hamrick 1984; Oostermeijer et al. 2003). Because of this influence, population biologists seek to understand how the mating system varies with population size and the consequences of such changes for population persistence. Studies demonstrate that offspring produced in small populations often exhibit lower germination, growth, and survival rates compared to offspring from larger populations (e.g., Menges 1991; Heschel and Paige 1994; Fischer and Matthies 1998a; Kéry et al. 2000). Such fitness changes call to mind the effects of inbreeding depression that can arise when inbreeding increases in small populations.

Self-fertilization can increase in small or low-density populations of self-compatible, entomophilous species in response to decreased pollinator visitation. Pollinator visitation is often reduced in such populations because pollinators are less attracted to small flowering patches (e.g., Sih and Baltus 1987; Jennersten 1988; Cunningham 2000). Some species compensate for reduced pollination through autonomous autogamy (i.e., self-pollination within a flower without the aid of pollinators) (Kalisz et al. 2004), but while this ability can buffer individuals against reproductive

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losses, it will increase the proportion of inbred progeny. Even when pollinators visit these small populations, changes in their foraging behavior can effect changes in the mating system. For example, pollinators are more likely to move among flowers within a plant rather than among plants when plant density is low (e.g., Heinrich 1979; Ghazoul et al. 1998; Field et al. 2005), potentially increasing geitonogamous selfing. Several studies have demonstrated increased selfing in small and/or low-density populations (e.g., Murawski and Hamrick 1991; van Treuren et al.1993; Routley et al. 1999; Hodgins and Barrett 2006). Moreover, this pattern is not restricted to entomophilous species; increased selfing has also been associated with reduced population size or density in wind-pollinated species (e.g., Farris and Mitton 1984; Robledo-Arnuncio et al. 2004).

Levels of inbreeding between relatives (i.e., biparental inbreeding) may also change with population size. Because individuals in small populations are more likely to be related to each other by chance than those in large populations, even random mating in small populations will lead to biparental inbreeding (Wright 1931). Consequently, one might expect a negative relationship between biparental inbreeding and population size. While this form of inbreeding has received less attention than selfing, it can nonetheless have similar fitness consequences (Heywood 1993; Nason and Ellstrand 1995). For example, Herlihy and Eckert (2004) found increased levels of biparental inbreeding in small populations of *Aqueligia canadensis* and suggested that this may be related to reduced reproduction in small populations.

In addition to increased inbreeding, small populations face the threat of reduced genetic diversity through genetic drift (Ellstrand and Elam 1993; Young et al. 1996; Frankel and Soulé 1981; Barrett and Kohn 1991). While genetic drift affects all populations, its effects—a reduction in genetic variation—manifest more rapidly in small populations (Wright 1931). For example, Paschke et al. (2002) found that small populations of the narrowly endemic perennial *Cochlearia bavarica* had fewer alleles per locus, fewer polymorphic loci, and reduced genetic diversity compared to larger populations. Similar patterns have been found in other species as well (e.g., van Treuren et al. 1991; Fischer and Matthies 1998b; Hensen and Oberprieler 2005; de Vere et al. 2009). Conservation biologists are concerned with the effects of genetic drift and inbreeding (i.e., reduced heterozygosity) because both of these effects may impact population viability through reducing individual fitness (Barrett and Kohn 1991; Ellstrand and Elam 1993) and/or diminish the population's ability to respond to selection (Gilpin and Soulé 1986; Booy et al. 2000). Positive relationships between genetic diversity and fitness and between observed heterozygosity and fitness have been found in a variety of species (e.g., Schaal and Levin 1976; Oostermeijer et al. 1995; Pluess and Stöcklin 2004; de Vere et al. 2009). However, the negative genetic effects of small population size may be mitigated by a variety of processes, including gene flow (Richards 2000) and selection against less fit, homozygous offspring and/or selection for more fit, heterozygous offspring (heterosis) (Farris and Mitton 1984; Charlesworth and Charlesworth 1987).

In this paper, we examine how the mating system and genetic diversity vary with population size across natural populations of *Sabatia angularis* L. (Pursh) (Gentianaceae), a biennial native to the eastern U.S. and Canada. *S. angularis* is common throughout much of its range but can often be found in patches with fewer than 20 individuals (Spigler and Chang 2008; Spigler 2007) and is currently at high risk of extinction in Kansas, Michigan, and New York (NatureServe 2009; USDA 2009). Currently there are no genetic estimates of outcrossing rates nor information on genetic diversity for *S. angularis*, but previous research has demonstrated that it experiences significant inbreeding depression (Dudash 1990; Spigler 2007), suggesting that variation in the mating system in small *S. angularis* populations could affect local population persistence. Therefore, we determined the outcrossing rate, biparental inbreeding rate, inbreeding coefficient, and genetic diversity, measured as allelic richness and expected heterozygosity, across eight natural *S. angularis* populations varying in size and evaluated whether these variables correlate with population size. We predicted that outcrossing rate and genetic diversity would increase with population size, whereas biparental inbreeding and the inbreeding coefficient would decrease with population size. Accordingly, we also predicted that observed heterozygosity would increase with population size. In addition, we compared inbreeding coefficients between the progeny and parental generation to gain insight into whether selection against inbred individuals occurs between generations (Ritland 1990). Finally, we examined how genetic diversity of this species is distributed by estimating F_{st}. Given the links among population size, genetic diversity, and fitness (Leimu et al. 2006), our results will be pertinent not only to *S. angularis*, but to improving our understanding of the effects of reduced population size on plant species in general.

Methods

Study species and sites

Sabatia angularis is an herbaceous obligate biennial that is widely distributed in a variety of habitats throughout its range in the eastern U.S. and Canada, including glades,

marshes, old fields, roadsides, prairies, and serpentine barrens. From July through August, plants produce showy, pink floral displays. Flowers are nectarless and offer only pollen as a reward to a suite of generalist pollinators that include leaf-cutter bees (Megachilidae), sweat bees (Halictidae), andrenid bees (Andrenidae), small carpenter bees (Anthophoridae), and hover flies (Syrphidae) (Dudash 1987; Spigler 2007). Upon pollination, flowers develop into many-seeded, dry dehiscent capsules that mature between September and November. Seedlings germinate in early spring and form rosettes that overwinter until the following spring when plants bolt.

Although previous research suggests that this species is outcrossing (Dudash 1987), several aspects of the species' biology suggest that significant inbreeding in natural populations might occur. *S. angularis* is fully self-compatible (Dudash 1987, 1990), and despite being protandrous, male and female phases often overlap, providing opportunities for autonomous self-pollination (autogamy) (Spigler, unpublished data). In addition, flowers in different phases are typically open at the same time within an individual. Given that a medium sized plant can have as many as 50 open flowers at a time, individuals may experience high levels of geitonogamy. Furthermore, seeds disperse by gravity from dehisced fruits that remain attached to the maternal plant, and such passive seed dispersal likely causes spatial family structure within populations (Wright 1943; Hamrick and Nason 1996), which, combined with local pollen dispersal (Levin and Kerster 1969), can lead to significant levels of biparental inbreeding (e.g., Zhao et al. 2009).

Natural populations of *S. angularis* vary in size, with the majority of populations ranging from 1 to 400 adult individuals in the region under study (Spigler and Chang 2008). Eight *S. angularis* populations located in North and South Carolina (Table 1; Fig. 1) were selected to represent the range of population sizes typical in that area. These populations were also included in a previous study on the relationship between population size and reproductive success (Spigler and Chang 2008). In July 2004, we assessed population size by counting all reproductive adults. Accurate counts of reproductive adults are feasible because natural *S. angularis* populations tend to be patchy and discrete. Distances between populations in this study ranged from 0.4 to 88 km, with a mean distance of 44.3 km (±29.8 SD) (median distance 35.9 km).

In each of the eight study populations, we randomly selected 20 focal individuals to serve as maternal plants. The only exception was population COW2, which initially consisted of only 15 flowering plants, but declined to 11 individuals. Prior to fruit dehiscence, we collected up to 15 naturally pollinated fruits from each focal maternal plant per population. In February 2005, we planted seeds in flats

Table 1 List of study populations including location and population size

Population	Location	Population size
COW2	Macon Co., NC	15
JRM	Clay Co., NC	55
LI1	Pickens Co., SC	57
BC2	Clay Co., NC	60
LI2	Pickens Co., SC	76
BC1	Clay Co., NC	207
WF	Macon Co, NC	220
WOM	Clay Co., NC	355
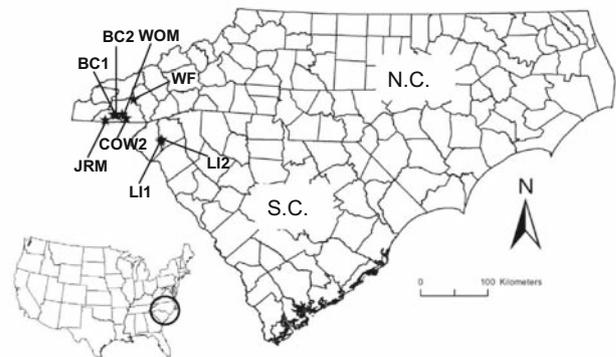
Fig. 1 Locations of *Sabatia angularis* study populations in North Carolina (NC) and South Carolina (SC), USA

at the University of Georgia Plant Biology Greenhouses and subjected them to cold stratification. For all planted seeds, we kept track of maternal plant and fruit identity. We grew the plants until the following January, when they were large enough to collect tissue for starch gel electrophoresis.

Enzyme extraction and electrophoresis

We used 8–15 seedlings per maternal individual per population for horizontal starch-gel electrophoresis. When possible, we used seedlings from different fruits to minimize sampling full sibs, which can affect the accuracy of mating system estimates (Schoen and Clegg 1984). We were able to sample seedlings from 6 to 15 fruits per family in approximately 70% of the cases. Even in the remaining 30%, however, the number of fruits sampled represented at least 40% of the total number of fruits produced by a maternal individual. We crushed approximately 400 mg of fresh tissue from each seedling in chilled mortars containing sand to grind the plant material and a polyvinylpyrrolidone-phosphate extraction buffer to extract enzymes (Mitton et al. 1979). Extracts were absorbed onto

Whatman 3 mm chromatography paper wicks and stored in microtiter plates at −70°C.

We examined eight polymorphic loci from the following enzyme systems: diaphorase (DIA1, DIA2), malic enzyme (ME2), fluorescent esterase (FE1, FE2, FE4), 6-phosphogluconate dehydrogenase (6-PGD3), and UTP-glucose-1-phosphate (UGPP3). Due to poor resolution, FE1 could not be scored accurately in a majority of individuals from populations LI1 and LI2, and thus this locus was not considered in the mating system analysis or calculation of genetic diversity statistics for these two populations. All stain recipes follow Soltis et al. (1983), except DIA (see Cheliak and Pitel 1984). The following enzyme and buffer system combinations were used: UGPP and 6-PGD with buffer system 4 (Tris–Citrate, pH 7.5) (Soltis et al. 1983) and ME, FE, and DIA with buffer system 8^- (concentrated LiOH) (modified from Soltis et al. 1983).

Data analysis

Outcrossing and biparental inbreeding rates

We estimated the outcrossing rate and biparental inbreeding rate for each population using the multilocus mating system program MLTR (Ritland 1990, 2002). Estimates of the multilocus outcrossing rate (t_m) and the mean single-locus outcrossing rate (t_s) were provided by MLTR. The multilocus estimate provides a more robust and accurate estimate of the population outcrossing rate. The single-locus estimate, however, is useful for calculating the biparental inbreeding rate (i.e., $m_b = t_m - t_s$) (Ritland 1990). We used default settings in MLTR for parameter estimations and generated 1,000 bootstrap estimates to obtain standard errors. Family, i.e., progeny array, was used as the resampling unit for bootstrap standard error construction (default option) (Ritland 2002). We ran MLTR separately for each population since allele frequencies were heterogeneous among populations (Ritland 2002). We calculated 95% confidence intervals for t_m and m_b as $1.96 \times$ SE and used these confidence intervals to evaluate whether t_m was significantly different from one (complete outcrossing) and whether m_b was significantly different from zero (no biparental inbreeding). We performed correlation analyses to evaluate whether these mating system parameters varied with population size.

Inbreeding coefficients

We calculated the inbreeding coefficient for the maternal generation (F_M) and the progeny generation (F_P). The inbreeding coefficient of the parental generation and associated standard errors were estimated in GenAlEx 6.1 (Peakall and Smouse 2006) on a per locus basis using

maternal genotypes inferred from progeny arrays in MLTR (Brown and Allard 1970). We calculated mean F_M across loci and 95% confidence intervals as $1.96 \times$ SE. For the progeny generation, we randomly sampled one offspring per maternal genotype using the software package R version 2.8.0 (R Development Core Team 2008) and calculated the progeny inbreeding coefficient (F_P) for each population as $1 - H_o/H_e$ for each locus and then averaged across loci. We ran 1,000 bootstraps in R, resampling one random progeny per maternal family, to obtain a robust estimate of mean F_P and corresponding 95% confidence intervals. We examined whether population size was correlated with F_M and F_P. We used the 95% confidence intervals to determine whether F_M and F_P were significantly different from zero; inbreeding coefficients less than or greater than zero would indicate an excess or deficiency of heterozygous individuals in the population, respectively, compared to Hardy–Weinberg expectations. We also evaluated whether the 95% confidence intervals for F_M and F_P overlapped to determine whether F_M was significantly lower than F_P, which might be expected if selection against homozygotes, and thus presumably inbred offspring, occurs between the two generations, assuming that the selfing rate and intensity of selection are constant among generations (Ritland 1990; Dole and Ritland 1993). Finally, we examined whether the absolute value of the change in F between generations (ΔF) was correlated with population size. Because this relationship was nonlinear (data not shown), we natural-log transformed population size.

Genetic diversity

To characterize genetic diversity in the *S. angularis* study populations, we estimated for each population: mean number of alleles per locus (A), mean effective number of alleles per locus (A_e), observed heterozygosity (H_o), and expected heterozygosity (H_e). We calculated these diversity statistics for the adult generation in each population using GenAlEx. We also calculated A, A_e, H_o, and H_e and corresponding 95% confidence intervals for the progeny in the software package R, using the 1,000 randomly generated progeny datasets described above. Because only polymorphic markers were utilized, the genetic diversity statistics presented are inflated relative to studies that combine monomorphic and polymorphic markers. Although this inflation makes comparisons between the genetic diversity of this species and other species difficult, it should not affect qualitative comparisons made among populations in this study. To evaluate whether genetic diversity was related to population size, we examined the correlation between A, A_e, H_o, H_e and population size. Because A and A_e were highly correlated in both the maternal and progeny generations (r for both = 0.94,

$P < 0.001$), as were H_o and H_e ($r = 0.91$–0.94, $P < 0.01$), we only present results from the correlation analysis for A to represent allelic diversity and H_e to represent heterozygosity.

Genetic differentiation among populations

We used AMOVA to determine how genetic diversity is distributed among populations (F_{st}) of *S. angularis*. As for the inbreeding coefficient and genetic diversity statistics, we calculated F_{st} based on maternal genotypes and the random subset of progeny genotypes. Analyses were performed using the hierfstat package in R (Goudet 2005). For the progeny, we calculated F_{st} for 1,000 randomly generated progeny datasets and obtained means and 95% confidence intervals. For both analyses, we included only the seven loci common to all study populations.

Results

There was a strong, positive relationship between outcrossing rate and population size among *S. angularis* populations (Fig. 2a). Mating systems of the eight populations ranged from mixed-mating (0.60 ± 0.08 SE) in population JRM to effectively complete outcrossing (0.95 ± 0.03 SE) in population WF. The mean outcrossing rate across study populations was 0.78 (± 0.04 SE).

There were significant levels of biparental inbreeding in all but two of the study populations, BC2 (95% CI for m_b: -0.041 to 0.065) and COW2 (95% CI for m_b: -0.050 to 0.110) (Fig. 2b, Supplementary Table 1). Mean biparental inbreeding across populations was 0.07 (± 0.01 SE). There was a positive correlation between the biparental inbreeding rate and population size, but it was not statistically significant (Fig. 2b).

Several trends were apparent from examining the inbreeding coefficients for progeny and maternal generations. First, progeny inbreeding coefficients (F_p) were positive for all populations, and in three populations (BC2, JRM, and LI2), F_p was significantly greater than zero based on 95% confidence intervals (Fig. 3a), indicating a significant excess of homozygotes in these populations. In contrast, F_M was negative in the majority of populations (Fig. 3a). For half of the populations (BC2, BW, COW2, and LI2), F_M was significantly less than zero (Fig. 3a), indicating a significant excess of heterozygotes. Across all populations, F_M was less than F_P, and for three populations (BC2, COW2, and LI2), this difference was significant (Fig. 3a). Correlation coefficients for the relationship between the inbreeding coefficient and population size were relatively strong for both progeny generations, and interestingly, they were in opposite directions, with a
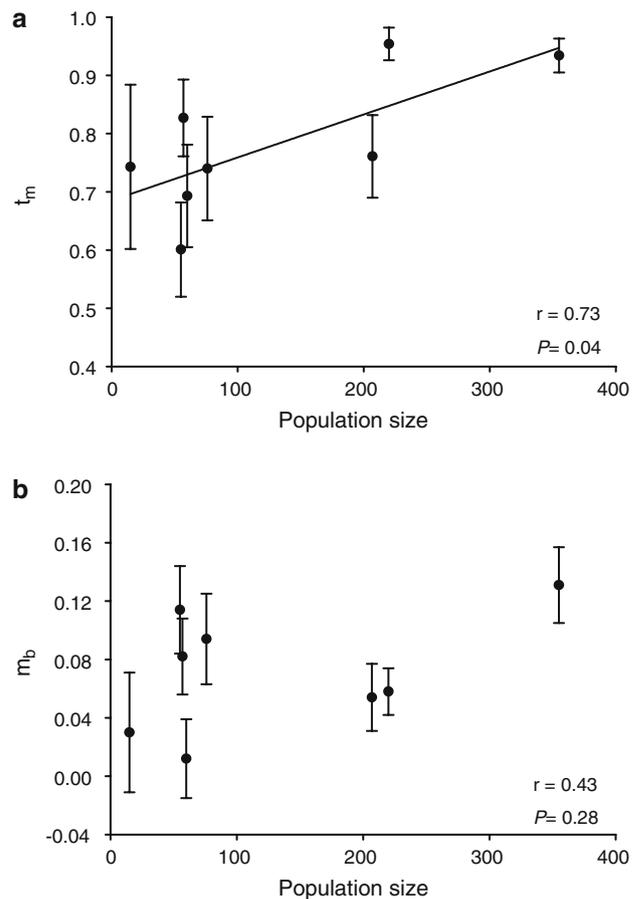
Fig. 2 Relationships between population size and **a** multilocus outcrossing rate (t_m) and **b** biparental inbreeding rate (m_b), measured as the difference between the multilocus and single locus outcrossing rates. *Regression lines* are shown for significant correlations to indicate the underlying trend

negative relationship for the progeny generation and a positive relationship for the maternal generation, although neither correlation was statistically significant (Fig. 3a). Consequently, there was a significant, negative relationship between ΔF, the change in inbreeding coefficients between generations (i.e., $|F_M - F_P|$), and population size (natural-log transformed) (Fig. 3b).

Estimates of genetic diversity for the maternal and progeny generations are presented in Table 2. The number of alleles, effective number of alleles, and expected heterozygosity were similar between the maternal and progeny generations. Consistent with the inbreeding coefficient results, observed heterozygosity was consistently greater in the maternal generation than in the progeny, and this was marginally significant for population LI2, where observed heterozygosity for the maternal generation was twice as high as that for the progeny. Neither allelic diversity based on the number of alleles per locus ($r_{Maternal} = 0.03$, $P = 0.94$; $r_{Progeny} = -0.07$, $P = 0.86$) nor expected heterozygosity ($r_{Maternal} = 0.01$, $P = 0.99$; $r_{Progeny} = 0.01$,
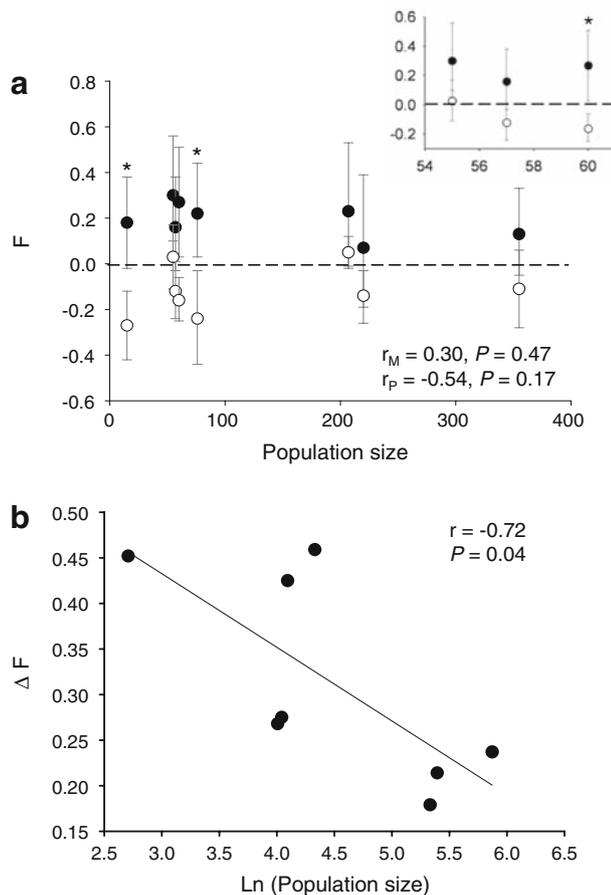
Fig. 3 Relationship between **a** the inbreeding coefficient (*F*) for the maternal (*open circles*) and progeny (*filled circles*) generations and population size and between **b** the change in *F* between generations (Δ*F*) and population size. In **a** correlation coefficients and corresponding *P*-values are presented for the maternal (r_M) and progeny (r_P) generations. *Bars* represent 95% confidence intervals. *Asterisks* indicate populations in which *F* is significantly different between the maternal and progeny generations. Values *above the dotted line* (*F* = 0) indicate an excess of homozygotes; those *below the dotted line* represent an excess of heterozygotes. *Inset* shows data for populations JRM, LI1, and BC2 at a magnified scale. In **b** correlation coefficient and *P*-value are presented, and a *regression line* is shown to indicate the underlying trend

P = 0.97) varied with population size, regardless of whether the maternal or progeny generation was examined.

We estimated F_{st} to be 0.27 and 0.34 (95% CI based on progeny: 0.30–0.38), for the maternal and progeny generations, respectively. These estimates indicate that approximately one-third of the total genetic variation in *S. angularis* is distributed among populations.

Discussion

Small populations are expected to experience increased levels of inbreeding and decreased genetic variation, two

consequences that can ultimately influence population viability. Whereas reductions in genetic diversity may not be seen for several generations, the effects of mating system change, although perhaps more subtle, can occur immediately following changes in population size. Our study clearly shows a strong, positive relationship between outcrossing rate and population size in natural populations of *Sabatia angularis* (Fig. 2). Approximately half of the variation in outcrossing rate among populations in our study populations was associated with variation in population size. The largest populations were almost completely outcrossed, whereas small populations had selfing rates as high as 40%, up to six times that in large populations. In fact, actual self-fertilization in natural populations may be underestimated given that early selection occurs against selfed progeny in these populations (Spigler 2007). Such high selfing rates in small *S. angularis* populations, combined with reduced reproduction (Spigler and Chang 2008) and significant inbreeding depression (Dudash 1990; Spigler 2007), could have severe consequences for population viability (Oostermeijer et al. 2003). These implications might include lower population growth rates and decreased extinction times, such as was found for populations of *Gentiana pneumonanthe* (Oostermeijer 2000).

Various hypotheses for the relationship between the mating system and population size involve changes in pollination quantity or quality with population size. For instance, evidence suggests that pollinator visitation rates decline in small populations (Sih and Baltus 1987; Jennersten 1988; Cunningham 2000; Knight et al. 2005). For many self-compatible species, this may not present an obstacle with respect to reproductive success if they are capable of autonomous autogamy (Kalisz et al. 2004). However, if the ratio of self to outcross pollen on stigmas declines with increases in pollinator visitation across population sizes in such autogamous species, we would expect to see a positive correlation between outcrossing rate and population size. *S. angularis* is capable of autogamy despite protandry (Spigler, unpublished data), and thus, increased selfing in small populations of *S. angularis* may reflect changes in the proportion of self pollen to outcross pollen deposited on stigmas. Similarly, Routley et al. (1999) concluded that this mechanism accounted for higher selfing rates in small populations of the self-compatible plant *Aquilegia canadensis*. Variation in geitonogamy among populations might also account for variation in the selfing rate if pollinators change their behavior in small populations. For example, pollinators increased intraplant movements relative to interplant movements in disturbed, low-density populations of a tropical tree (Ghazoul et al. 1998). Experimental floral manipulations involving pollinator exclusion and emasculation treatments will be used in future studies to discriminate between the quantities of

Table 2 Genetic diversity statistics for each population for maternal and progeny generations

Population	A	A_e	H_e	H_o
Maternal plants				
COW2	2.13	1.49	0.27	0.38
JRM	2.25	1.48	0.27	0.26
LI1	2.57	1.62	0.30	0.36
BC2	1.75	1.29	0.19	0.22
LI2	2.57	1.74	0.40	0.51*
BC1	1.88	1.32	0.18	0.16
WF	2.25	1.47	0.25	0.31
WOM	2.38	1.62	0.33	0.37
Mean	2.22 ± 0.30 SD	1.50 ± 0.15 SD	0.27 ± 0.07 SD	0.32 ± 0.11 SD
Progeny				
COW2	1.93	1.41	0.24	0.21
JRM	1.90	1.43	0.24	0.16
LI1	2.17	1.49	0.26	0.21
BC2	1.73	1.21	0.14	0.10
LI2	2.34	1.62	0.34	0.27*
BC1	1.71	1.29	0.16	0.12
WF	1.82	1.38	0.20	0.21
WOM	2.09	1.55	0.29	0.26
Mean	1.96 ± 0.22 SD	1.42 ± 0.13 SD	0.24 ± 0.07 SD	0.19 ± 0.06 SD

Populations are listed from smallest to largest

Means across all populations for each generation and standard deviations are given

Variables are calculated per locus, and mean values across loci are presented

A Mean number of alleles/locus, A_e mean effective number of alleles/locus, H_o mean observed heterozygosity, H_e expected heterozygosity

* Significant differences between the generations based on confidence intervals

outcross, autogamous, and geitonogamous pollen loads on *S. angularis* individuals in small and large populations and thus to test these hypotheses.

We found significant biparental inbreeding in almost all study populations, which likely reflects both localized seed and pollen dispersal. Seeds of *S. angularis* disperse passively from fruits that remain attached to maternal plants, and most seeds remain near the maternal plant (Dudash 1991). Furthermore, a majority of pollinator flights between plants of *S. angularis* occur within 1 m of a given plant (Dudash 1987, 1991). Because neighboring individuals in small populations should be related to each other by chance more so than in a large population, we predicted that biparental inbreeding would increase with decreasing population size. However, we did not detect a statistically significant relationship between these two parameters, and in fact, there was a trend toward increasing, not decreasing, biparental inbreeding with increasing population size. Undoubtedly, our statistical power was restricted because of our limited sample of populations. Yet despite this restriction, we could detect a strong relationship between outcrossing rate and population size. Thus, it is more likely that factors other than population size are more important

in influencing biparental inbreeding, such as density, which can influence the degree of overlapping seed shadows and pollen carryover (Levin and Kerster 1969; Harder and Barrett 1996).

Examination of the inbreeding coefficients for progeny and maternal generations most noticeably revealed that the maternal generation had less inbreeding than the progeny in all populations examined and that there was a significant negative relationship between the change in the inbreeding coefficient between generations (ΔF) and population size (Fig. 3). van Geert et al. (2008) also found higher inbreeding levels in the seedling generation compared to the adult generation in fragmented populations of *Primula vulgaris*, possibly due to increased selfing, inbreeding depression, heterosis, or overlapping adult generations. In our study, selfing in the generation under study but not in the previous generation that produced the maternal individuals could explain why we found higher inbreeding coefficients in the progeny generation. Similarly, the negative relationship between ΔF and population size might be explained alone by increased selfing in smaller populations in the generation under study. These explanations, however, require that the reductions in population size and

corresponding changes in the mating system represent very recent events in this biennial species. More interestingly and perhaps likely, selection against inbred, homozygous individuals between seedling and adult stages could be causing these patterns, assuming that selfing rate and selection intensity are the same across generations (Ritland 1990; Dole and Ritland 1993). *S. angularis* has been shown previously to exhibit significant inbreeding depression (Dudash 1990), and more specifically, we know that there is significant inbreeding depression in our study populations (Spigler 2007; Spigler, unpublished data). Selection against inbred offspring would also account for the unexpected result that maternal inbreeding coefficients were not just lower than the progeny inbreeding coefficients but actually negative in most study populations, indicating an excess of heterozygotes. Together, our results point to the intriguing possibility that the negative relationship between ΔF and population size is due to increased selection for outcrossed offspring or increased inbreeding depression in small populations.

In contrast to our initial prediction, genetic diversity was not related to population size in the *S. angularis* study populations. Our results suggest that even if we increased the number of populations studied, the relationship between genetic diversity and population size in *S. angularis* would not be biologically significant. The apparent independence between genetic diversity and population size contrasts with the current paradigm that small populations should have lower observed heterozygosity due to inbreeding and reduced allelic diversity via genetic drift (Frankel and Soulé 1981; Barrett and Kohn 1991; Ellstrand and Elam 1993). A recent meta-analysis examining over 40 independent empirical studies concluded that, indeed, positive relationships between genetic diversity and population size are the rule rather than the exception (Leimu et al. 2006). A persistent seed bank (Levin 1990; McCue and Holtsford 1998) or gene flow (Richards 2000) could have buffered the loss of genetic diversity in small populations of *S. angularis*. AMOVA revealed that F_{st} for this species, at least in the geographic range included in this study, is approximately 0.30, which is typical of other monocarpic, gravity-dispersed species with a mixed mating system (Hamrick and Godt 1996). This level of genetic differentiation is fairly high and suggests that gene flow between *S. angularis* populations is not high enough to counteract genetic drift. Alternatively, we might not see the expected relationship between genetic diversity and population size if the current population sizes reflect recent events. One caveat in our interpretations is that the size range of populations in this study, although representative of natural populations found in the study region, may not have been sufficient to capture the relationship between genetic diversity and population size. Preliminary evidence

from a rare extremely large population ($\sim$7,000 individuals), however, suggests that increasing the population size range might not affect our results. Expected heterozygosity in that population (0.27 ± 0.07 SE) was equivalent to the mean expected heterozygosity seen across the current study populations (Table 2) (Spigler, unpublished data).

The results of this study join others in demonstrating a positive relationship between the outcrossing rate and population size (e.g., Raijmann et al. 1994; Robledo-Arnuncio et al. 2004; Hodgins and Barrett 2006). Moreover, by examining the inbreeding coefficient in two generations and genetic diversity, we can make some preliminary predictions about the genetic consequences of reduced population size in a species with a mixed-mating system and inbreeding depression such as *S. angularis*. An increase in the selfing rate in small populations combined with lowered survival of offspring may translate into lowered population growth rates and increased risk of extinction. On the other hand, an excess of heterozygotes across populations in the maternal generation in our study and the absence of a relationship between observed heterozygosity and population size suggests that inbreeding depression may actually preserve population fitness in small populations by purging the population of its genetic load. Thus, the ultimate consequences for the viability of small populations will depend on the balance between the population benefits of purging the genetic load and demographic cost of selection. Future work will investigate the links among the mating system, inbreeding depression, and population viability in this species.

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