

Pollen limitation and reproduction varies with population size in experimental populations of *Sabatia angularis* (Gentianaceae)

Rachel B. Spigler and Shu-Mei Chang

Abstract: Individuals in large plant populations are expected to benefit from increased reproductive success relative to those in small populations because of the facilitative effects of large aggregations on pollination. As populations become small, the inability to attract sufficient numbers of pollinators can reduce reproduction via pollen limitation. This study experimentally tested whether such trends occur for the herbaceous biennial *Sabatia angularis* (L.) Pursh (Gentianaceae). We created artificial populations of varying size consisting of potted *S. angularis* plants in two field sites to determine whether population size affected mean fruit and seed set. We also examined whether population size affected the degree of pollen limitation using a supplemental pollination design in one of the sites. Our results showed that, on average, seed set was lower in large populations, not small populations, of *S. angularis* and that this result may be due to increased pollen limitation in large populations. We suggest that in certain contexts, small populations may enjoy reproductive advantages over large populations by escaping intraspecific competition for pollinators.

Key words: competition, facilitation, pollen limitation, population size, reproduction, seed set.

Résumé : On s'attend à ce que les individus de grandes populations bénéficient d'un succès reproductif relatif plus grand que ceux des petites populations dû aux effets encourageants d'une forte agrégation de pollen. À mesure que les populations deviennent petites, l'incapacité d'attirer des nombres suffisants de pollinisateurs pourrait réduire la reproduction par une limitation du pollen. Dans cette étude expérimentale, les auteurs ont vérifié si de telles tendances existent chez l'espèce herbacée *Sabatia angularis* (L.) Pursh (Gentianaceae). Ils ont créé des populations artificielles de diverses dimensions constituées de plantes en pot du *S. angularis* dans deux sites de prairie, afin de déterminer si la dimension des populations affecte les moyennes de fruits produits et de graines formées. Ils ont également vérifié si la dimension des populations affecte le degré de limitation par le pollen en utilisant une pollinisation supplémentaire sur un des sites. Les résultats montrent qu'en moyenne la formation des graines est plus faible dans les grandes populations, et non dans les petites, du *S. angularis* et que ce résultat pourrait provenir d'une augmentation de la limitation par les pollens dans les grandes populations. Les auteurs suggèrent que dans certains contextes, les petites populations possèdent des avantages reproductifs par rapport aux grandes populations en échappant à la compétition intra spécifique pour les pollinisateurs.

Mots-clés : compétition, facilitation, limitation par le pollen, dimension des populations, reproduction, formation des graines.

[Traduit par la Rédaction]

Introduction

Reduced reproduction in small plant populations is becoming a widely recognized phenomenon (Leimu et al. 2006), and has primarily been attributed to reductions in the quantity and quality of pollination services. For example, pollinator visitation is often reduced, as pollinators tend to

be less attracted to small populations than large ones. As a result, individuals in small populations may fail to receive enough pollen to achieve full fruit or seed set (Sih and Baltus 1987; Aizen and Feinsinger 1994; Ågren 1996). Thus, the expectation is that individuals in small populations are more likely to be pollen limited than their counterparts in large populations, and this can become exacerbated if individuals in small populations must also compete with other species for pollinator visitation (Levin and Anderson 1970; Rathcke 1983). This competition can negatively affect the quantity of pollen received, e.g., via reduced visitation or reduced conspecific pollen carryover and deposition (Caruso 1999; Brown et al. 2002; Bell et al. 2005), and can also affect pollen quality via the transfer of heterospecific pollen (Waser 1978a, 1978b; Campbell and Motten 1985; Galen

Received 26 August 2008. Published on the NRC Research Press Web site at botany.nrc.ca on 6 March 2009.

R.B. Spigler^{1,2} and S.-M. Chang. Department of Plant Biology, University of Georgia, Athens, GA 30602, USA.

¹Corresponding author (e-mail: rbs12@pitt.edu).

²Present address: Department of Biological Sciences, 4249 Fifth Avenue, University of Pittsburgh, Pittsburgh, PA 15260, USA.

and Gregory 1989), which can also result in reduced reproduction. Furthermore, small populations may face reduced pollination because they are unable to sustain sufficient pollinator populations (Bowers 1985; Potts et al. 2004; Moeller 2005). Even if pollination services do not change, small populations are often confronted by reduced genetic diversity, via drift or inbreeding (Frankel and Soulé 1981; Barrett and Kohn 1991; Young et al. 1996), and such reductions have also been associated with lowered reproductive success (Reed and Frankham 2003; Leimu et al. 2006). These patterns suggest that facilitative intraspecific interactions may be important for successful reproduction in natural plant populations. This phenomenon is often referred to as the Allee effect (Allee 1931; Allee et al. 1949), and has been demonstrated empirically in several species (e.g., Lamont et al. 1993; Widén 1993; Groom 1998; Kéry et al. 2000; Hackney and McGraw 2001).

Yet despite these trends, reproduction need not always be reduced in small populations. Some species, for example, require only a small amount of pollen to set seed such that their reproductive success is unaffected by changes in population size and pollinator visitation (Molano-Flores et al. 1999). Other species may be able to mitigate the consequences of reduced pollinator visitation through autonomous autogamy (self-pollination within a flower) (Karoly 1992; Kalisz et al. 2004), although this ability may not secure a plant's fitness if resultant fruit or seed set is reduced by inbreeding depression (Stephenson 1981; Stephenson and Winsor 1986). Reproduction in small populations might also be sustained when other flowering species facilitate pollinator visitation, rather than compete (Rathcke 1983; Callaway 1995; Ghazoul 2006). Positive interactions between species for pollinators are less documented than competitive interactions, but occur (e.g., Waser and Real 1979; Moeller 2004; Ghazoul 2006) and are becoming commonly recognized (Brooker et al. 2008). Furthermore, reproduction may even be greater in small populations than in large ones, if individuals in small populations are released from competition for resources (Harper 1977). Limited resource availability can restrict fruit and seed set regardless of the amount of pollen received (Haig and Westoby 1988; Ashman et al. 2004). For example, Mustajärvi et al. (2001) found that large populations of the perennial herb *Lychnis viscaria* had lower fruit set than small populations despite similar levels of pollination.

Observational studies on the relationship between population size and reproduction in natural populations have been essential in identifying relevant patterns in nature, but they unavoidably confound the potential mechanisms affecting variation in reproduction, including resource availability, pollen limitation, and other ecological factors. Experimental studies examining these relationships are useful for isolating these factors and for identifying the specific mechanisms that influence the relationship between population size and reproduction. As such, experimental studies can add to our understanding of how reproductive dynamics change with population size, knowledge that is useful for improving our understanding of population dynamics in general.

The goal of this study was to examine the effect of population size on reproductive success in the native biennial *Sabatia angularis* (L.) Pursh (Gentianaceae). Previous research

in natural *S. angularis* populations has shown that fruit set is reduced in small populations (Spigler and Chang 2008) but was unable to positively conclude whether this effect was pollinator mediated. *Sabatia angularis* individuals are known, however, to experience pollen-limited fruit and seed set (Dudash 1993). Thus in this study we use an experimental approach designed to isolate the effect of population size on reproduction and to evaluate the relationship between population size and pollination. Specifically, we created artificial populations consisting of 1, 4, 25, 100, and 225 individuals using potted *S. angularis* plants, and asked the following questions: (i) Do small populations of *S. angularis* have reduced fruit and seed set? (ii) Is reproduction in these populations pollen limited? (iii) Is the degree of pollen limitation greater in small populations compared with larger populations?

Materials and methods

Study species

Sabatia angularis is an herbaceous biennial native to eastern North America. Widely distributed throughout its range, it may be found in a variety of habitats including glades, marshes, rocky outcrops, old fields, roadsides, and prairies. The majority of plants typically produce their pink flowers from July until August. Flowers are protandrous and self-compatible, and last approximately 4 d (Dudash 1987), although they can last over a week if unpollinated (R.B. Spigler, personal observation). Despite protandry, individuals are capable of autogamous pollination when male and female phases overlap within a flower (R.B. Spigler, unpublished data). The flowers are nectarless, and instead offer pollen as a reward to a suite of generalist pollinators, which may include leaf-cutter bees (Megachilidae), sweat bees (Halictidae), andrenid bees (Andrenidae), small carpenter bees (Anthophoridae), and hover flies (Syrphidae) (Dudash 1987; Spigler 2007). Between August and November, flowers develop into many seeded, dry, dehiscent capsules from which seeds mainly disperse passively by gravity. Seeds germinate the following spring and develop into a rosette by late fall.

Plant cultivation and experimental arrays

In January 2005 in the Plant Biology Greenhouse at the University of Georgia, we planted approximately 1000 *S. angularis* seeds that were collected the previous reproductive season from 10 natural populations across North Carolina, South Carolina, and Georgia. We subjected planted seeds to cold stratification to promote germination, and once rosettes developed in Fall 2005, we transplanted them into 6-inch (1 in. = 25.4 mm) Regal Azalea pots (Hummert International, Springfield, Mo.) filled with a 1:1 v/v pine-bark soil – turface MVP (Profile, Buffalo Grove, Ill.) mixture. The pine-bark soil contained vermiculite and fertilizer ($\text{Ca}(\text{NO}_3)_2$, KNO_3 , gypsum, limestone, and Micromax (Scotts International B.V., Geldermalsen, the Netherlands)). Most plants bolted and began to develop flower buds by June 2006.

Two replicates of the experiment were performed across two sites: Whitehall Forest, an 800 acre tract (1 acre = 0.4 ha) of Piedmont forest managed by the Warnell School of Forestry and Natural Resources at the University of Geor-

gia in Athens, Georgia, and a large, open meadow in Farmington, Georgia. While *S. angularis* has been found previously in these parts of Georgia, the species is not common here, and no local populations of *S. angularis* were located near the study sites. Individuals were assigned to one of five population size classes: $N = 1, 4, 25, 100,$ and 225 (see below). We chose experimental population size classes to represent natural variation in population size of contemporary wild *S. angularis* populations in the region; natural populations in Georgia, North Carolina, and South Carolina are patchy and typically vary in size from 1 to 400 adult plants (Spigler and Chang 2008). Although larger populations, up to ~ 7000 plants, do exist in this region, they are rare and found only in managed prairies (R.B. Spigler, personal observation).

Because flowering among the experimental plants grown in the greenhouse was not synchronous and spanned over a period of 2 months, we assigned individuals to a population size class and site based on flowering time. We were able to determine which plants would flower approximately at the same time according to the stage of flower buds of plants in the greenhouse prior to placing them in the field. By July 2006, approximately half of the plants raised in the greenhouse had large, mature, pink floral buds, while floral buds were still small and whitish-green in the remaining plants at that time. Among those plants with mature flower buds, we randomly assigned individuals to a population size class and placed them in arrays at Whitehall Forest (see below). Among the remaining individuals, we waited until floral buds were mature (approx. 3 weeks later) and at that time randomly assigned those individuals to a population size class and placed them in arrays at the Farmington site. This design ensured that flowering was approximately synchronous among plants within the same array and among arrays within each site. Because individuals were assigned randomly with respect to source population, we were also able to eliminate genetic structure among populations.

Experiment 1: Population size and reproduction

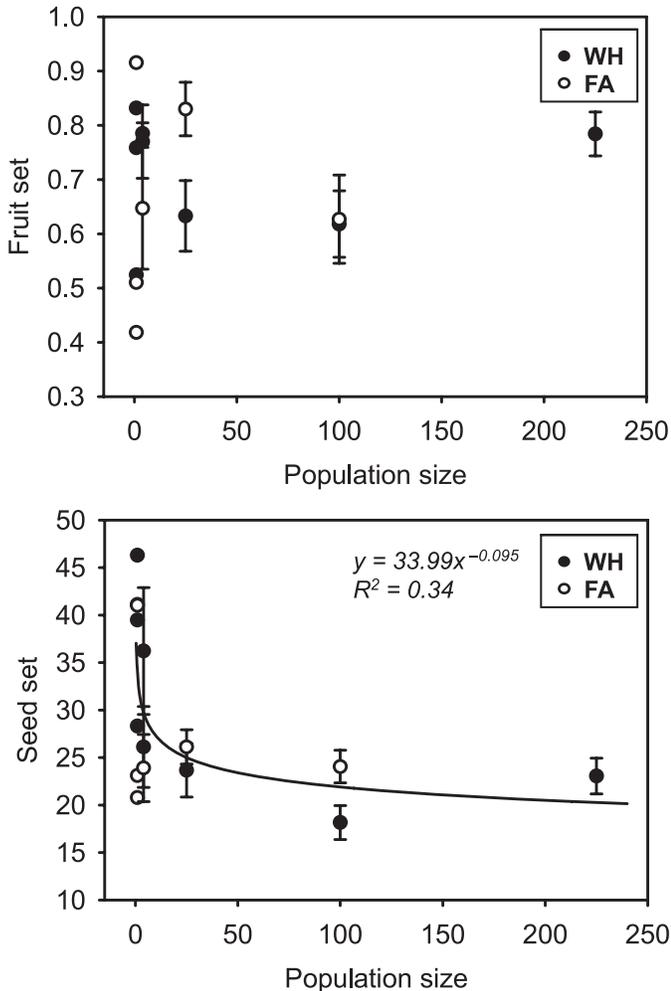
The first replicate began July 2006 at Whitehall Forest (hereinafter, WH). Within the forest matrix of WH, we located open fields and power-line right-of-ways and randomly assigned each artificial population to a given location. These locations were separated by at least 500 m. Plant arrays consisted of potted plants placed 0.5 m apart in a square grid to keep density constant. We included four replicates of $N = 1$, two replicates of $N = 4$, and one replicate each of $N = 25, 100,$ and 225 within WH. To keep plants alive, we watered plants daily throughout the duration of the study. In each of the $N = 25, 100,$ and 225 population size classes, we randomly selected 15 individuals as target plants; all individuals were included in smaller populations. For each of these target plants, we counted the number of flower buds. The experiment ended when focal plants had finished flowering and fruits had matured. At that time, we counted the number of fruits to measure proportion fruit set and we collected fruits to estimate seed set. Because capsules remain attached to the plant, it is highly unlikely that any fruits were missed. We measured fruit set as the ratio of fruit number to bud number. Because *S. angularis* seeds are so small (approx. 0.025 mg/seed, on average), we used fruit mass as a proxy

for seed set. Fruit mass is significantly and highly correlated with total seed mass per fruit ($r = 0.985, P < 0.0001, n = 71$), and total seed mass is significantly correlated with seed number ($r = 0.88, P = 0.003, n = 11$) (R.B. Spigler, unpublished data). We estimated seed set as average fruit mass across up to 10 fruits per individual. After plants began to flower, a host-specific fungus, identified as *Cercospora sabbatae*, spread throughout the experimental populations in site WH, and many plants became infected. Therefore, we also recorded presence or absence of infection for each target plant.

In August 2006, we brought potted plants to the second site in Farmington, Georgia (hereinafter, FA). Within FA, we placed three replicates of $N = 1$ and one replicate each of $N = 4, 25,$ and 100 . The reduced number of replicates and exclusion of $N = 225$ resulted from a limited number of plants with mature buds in the greenhouse by that time. Because FA is much smaller in area than WH, the distance between artificial arrays was reduced to approximately 150 m. We watered plants daily and randomly selected up to 15 target plants in each experimental array to collect data as described above. In addition, once arrays were established at FA, we sprayed all plants with a fungicide (1 tsp./gallon (1 tsp. = 4.9 mL, 1 gallon = 3.78 L) of Cleary's 3336 WP (Cleary Chemical Corp., Dayton, N.J.) with 0.5 tsp./gallon Bio 88 surfactant (KALO, Overland Park, Kansas, USA)) in response to widespread infection of WH experimental plants. Fruit and seed set were assessed once focal plants ceased flowering and fruits had matured.

All analyses, including those for experiment 2 (see below) were performed in SAS (SAS Institute Inc. 1999, version 9.1). To determine whether the relationship between population size and either fruit or seed set differed between the two sites, we examined the effect of population size, included as a continuous variable, site, and their interaction on mean population fruit and seed set in ANOVA (PROC GLM). Seed set was natural-log transformed to conform to ANOVA assumption of normality. A significant interaction between site and population size would indicate that the relationship between population size and either fruit or seed set differed among sites. This interaction was not significant for either fruit set ($F_{[1,13]} = 0.00, P = 0.99$) or seed set ($F_{[1,14]} = 0.02, P = 0.65$), therefore we proceeded by pooling the data from both sites and examined the relationship between population size and fruit and seed set using regression analysis (PROC GLM). Initial examination of scatter plots suggested that the relationship between seed set and population size may, in fact, be nonlinear (Fig. 1). To evaluate this alternative, we also fit the pooled seed set data to the asymptotic power function $y = ax^b$ (PROC NLIN) and calculated the R^2 for this function as $1 - (SSE/SST_{\text{corrected}})$, where SSE is the error sum of squares and $SST_{\text{corrected}}$ is the corrected total sum of squares (Kvålseth 1985). We then calculated residuals from the pooled nonlinear relationship and used ANOVA to determine whether there was significant variation between sites in their deviation from the pooled nonlinear relationship (Waser and Price 1991; Mitchell 1997). ANOVA revealed that the two sites did not differ in their fit to this model ($F_{[1,14]} = 2.26, P = 0.16$), suggesting again that the sites do not vary in their response; therefore we refer only to the pooled data for this analysis. Pooling of data was

Fig. 1. Mean fruit set (top panel) and seed set (bottom panel), measured as mean fruit mass per plant (in mg), for experimental *Sabbatia angularis* populations of varying size: ●, Whitehall (WH); ○, Farmington (FA). The significant nonlinear relationship between population size and seed set based on the pooled data is represented as a solid line, and the equation describing this relationship is presented. Standard error bars around mean values are shown.



also supported by comparison of parameter estimates from site-specific nonlinear models, which revealed overlapping 95% confidence intervals for these parameters (R.B. Spigler, unpublished data). We compared R^2 values from the competing linear and nonlinear models to evaluate which model better described the relationship between seed set and population size.

We performed a second set of analyses to ensure that our results were not artifacts of incidental fungal infection, which occurred at site WH. We first calculated adjusted fruit and seed set means for population arrays at WH that accounted for the effects of the fungal pathogen. Adjusted means were obtained from the LSMEANS statement in PROC GLM, wherein fruit and seed set were modeled as a function of population size array (used as a class variable) and presence or absence of fungal infection. We then repeated the analyses described above, substituting the adjusted means for population arrays at WH in the pooled data set. Data were also pooled for these analyses because

there was not a significant site by population size interaction for fruit set ($F_{[1,13]} = 0.02$, $P = 0.90$) nor for seed set ($F_{[1,14]} = 0.11$, $P = 0.75$), nor did sites differ significantly in their fit to pooled nonlinear relationship based on adjusted means ($F_{[1,14]} = 0.88$, $P = 0.37$).

Experiment 2: Population size and pollen limitation

To determine whether reproduction is pollen limited and whether the degree of pollen limitation varies with population size, we performed a pollen supplementation experiment at WH. We randomly selected 10 plants in each of the $N = 25$, 100, and 225 size classes and 2 plants in one of the $N = 4$ replicates to serve as “treatment” plants and paired them with “control” plants in the same population array that were similar in height, bud number, and flowering time. Control plants were a subset of the 15 target plants used in experiment 1. All flowers per treatment plant received supplemental pollen; each day we applied pollen to every flower with a receptive stigma by directly rubbing anthers onto the stigmatic surface until visibly covered with the yellow pollen. The majority of supplemental hand pollinations were carried out between 0800 and 1100 h. The supplemental pollen represented outcross pollen, which we chose haphazardly from plants in the array that had available pollen each day and that were not used as target plants in experiment 1 or treatment or control plants in this experiment. Because all of the arrays are composed of a mixture of plants from the 10 original seed source populations, crosses may be between individuals originating from the same population or between individuals originating from different populations. Previous data, however, revealed that neither population identity of the pollen donor nor the recipient has a significant effect on fruit or seed set (R.B. Spigler, unpublished data). We estimated fruit and seed set for treatment and control plants as in experiment 1.

We performed paired t tests (PROC TTEST) to evaluate whether treatment plants receiving supplemental pollen had significantly higher fruit and seed set than control plants across all population sizes, which would suggest that reproduction was, on average, pollen limited. Because some treatment and control plants were infected by *C. sabbatae*, we repeated the paired t tests, including only those pairs that had either no or minimal fungal damage, to remove potential confounding effects of this infection.

To determine whether the degree of pollen limitation was related to population size, we first estimated the degree of pollen limitation using the pollen limitation index $L = 1 - (P_o/P_s)$, where P_o is fruit or seed set of open-pollinated controls and P_s is the fruit or seed set of treatment plants given supplemental pollen (Larson and Barrett 2000). We calculated mean L for each population size class and used correlation analysis to examine whether population size was significantly related to pollen limitation (PROC CORR).

Results

Experiment 1: Population size and reproduction

There was a significant, negative effect of population size on seed set, and the relationship between the two was nonlinear ($F_{[2,15]} = 119.81$, $P < 0.0001$, $R^2 = 0.34$) (Fig. 1). This

nonlinear relationship remained significant when mean seed set for populations at WH was adjusted for fungal infection ($F_{[2,15]} = 127.52$, $P < 0.0001$, $R^2 = 0.29$). The linear model describing the effect of population size on seed set was not significant ($F_{[1,14]} = 3.10$, $P = 0.10$, $R^2 = 0.19$). While the relatively low P value from the linear model suggests that we may not be able to detect a linear effect simply because of low power, it nevertheless explained only 19% of the variation in seed set compared with 34% explained by the nonlinear model. This conclusion did not change when adjusted seed set values were used in the linear model ($F_{[1,14]} = 2.41$, $P = 0.15$, $R^2 = 0.16$). In contrast with the results for seed set, population size did not significantly affect fruit set ($F_{[1,13]} = 0.09$, $P = 0.77$) (Fig. 1), and this result did not change when fungal infection was accounted for ($F_{[1,13]} = 0.32$, $P = 0.58$). We did not detect a significant effect of site in the initial ANOVA on fruit set ($F_{[1,13]} = 0.27$, $P = 0.61$) or seed set ($F_{[1,14]} = 1.96$, $P = 0.19$) (see also Methods for nonlinear).

Experiment 2: Population size and pollen limitation

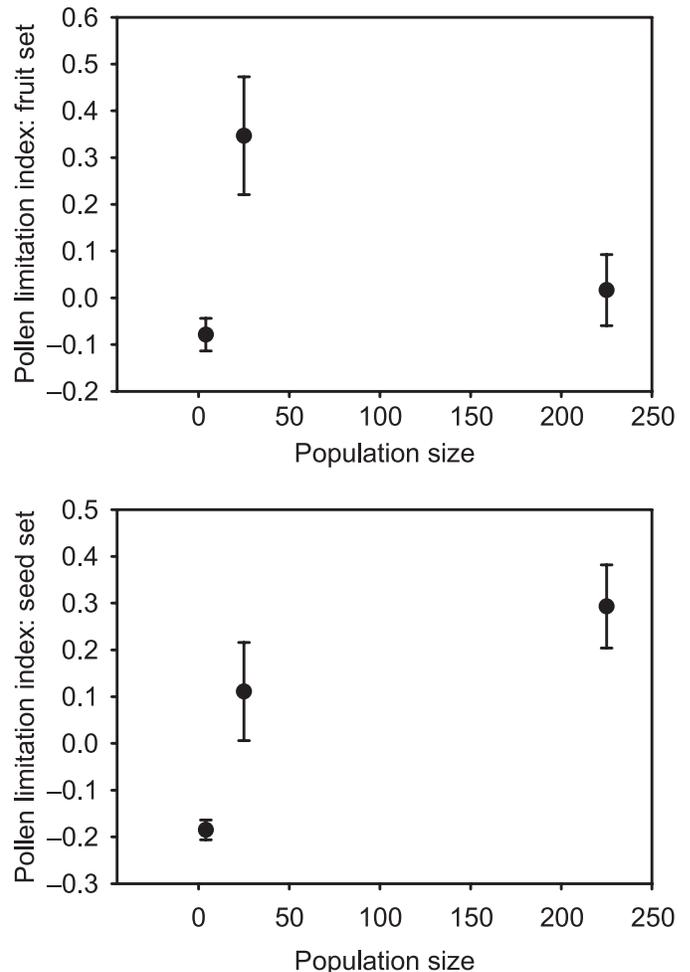
An initial paired t test revealed no significant difference between pollen-supplemented and control plants for fruit set ($t = 0.95$, $df = 28$, $P = 0.35$) or seed set ($t = 0.01$, $df = 30$, $P = 1.0$). When the analyses were performed involving only uninfected, healthy individuals, a trend emerged: plants receiving supplemental pollen had significantly greater seed set than control plants across population size classes ($t = 2.13$, $df = 14$, $P = 0.05$). Fruit mass, our proxy for seed set, was on average 5.77 mg greater in pollen-supplemented plants than control plants. Among healthy plants, fruit set was also marginally greater in treatment plants than control plants ($t = 1.99$, $df = 13$, $P = 0.07$), with pollen-supplemented plants setting approximately 15% more fruits.

We proceeded by determining whether the degree of pollen limitation among healthy plants varied with population size. Because almost all individuals in $N = 100$ were affected by the fungus, this population size class was omitted from the analysis. Unfortunately, this left only three population size classes for correlation analysis. Examination of the relationship between population size and pollen limitation revealed a trend of increasing pollen limitation for seed set with population size (Fig. 2), and the correlation between population size and pollen limitation was positive and high ($r = 0.84$), but not surprisingly, given the low power, was not statistically significant ($P = 0.37$). While a Spearman correlation based on ranks does support this relationship ($\rho = 1.0$, $P < 0.0001$), the relationship might also be nonlinear (Fig. 2), but again low power precludes further examination. There was no relationship between population size and pollen limitation for fruit set ($r = -0.23$, $P = 0.85$) (Fig. 2).

Discussion

The goal of this study was to isolate the effect of population size on reproduction in *Sabatia angularis* and to evaluate whether this effect was pollinator mediated. We found that population size affected reproduction, but interestingly, this effect was in the opposite direction that we predicted. We initially predicted that small populations would experience reduced reproduction because of reduced pollinator vis-

Fig. 2. Degree of pollen limitation for fruit set (top panel) and seed set (bottom panel), estimated using the pollen limitation index L , plotted against population size. Only healthy plants are included in the data set. Standard error bars around mean values are shown.



itation and subsequent increased pollen limitation, as has been found in other species. For example, Ågren (1996) demonstrated that increased pollen limitation in small populations of the tristylous herb *Lythrum salicaria* led to a positive relationship between seed set and population size. Similarly, Knight (2003) found increased pollen limitation in sparse populations of the understory herb *Trillium grandiflorum* compared with dense populations. These studies support the emerging general principle that facilitative interactions among individuals for increased pollination are important for successful reproduction (Leimu et al. 2006).

Such facilitative effects, however, were not apparent in this study. Instead, there was a negative effect of population size on reproduction, and large populations had lower seed set than small populations. This effect was nonlinear, such that mean population seed set declined rapidly with initial increases in population size, but the rate of this decline lessened with further increases in population size (Fig. 1). Based on the nonlinear model fit to the data, this relationship translates into a 20% decline in mean population seed set between a population of only 1 individual and a population of 10 individuals, for example, but when comparing popula-

tions of 100 and 225 individuals, this decline is reduced to 8%. Although reduced, an 8% decline in mean seed set per fruit might nevertheless be expected to have a significant impact on individual fitness.

Reduced reproduction in large populations often arises as an outcome of increased competition for soil resources with increasing population size (Harper 1977). Yet in this study, the use of individually potted plants excluded the role of plant–plant competition for soil resources. Instead, the effect of population size on seed set seen in this study may have been pollinator mediated. This possibility is consistent with results from experiment 2, which revealed that individual reproduction was pollen limited and that, at least for seed set, the degree of pollen limitation tended to increase with population size, although we note the small sample size precludes statistical confirmation of this relationship. The levels of pollen limitation seen for seed set in the $N = 25$ and $N = 225$ size classes examined here correspond well with a previous report of pollen limitation in a natural population of *S. angularis* (Dudash 1993), but studies on the relationship between pollen limitation and population size in natural populations do not exist for this species, limiting further comparison. Rather than compete for below-ground resources, *S. angularis* individuals in this experiment may have competed for pollinators. Preliminary pollinator observations at WH indicated few visitors to plants in experimental populations (R.B. Spigler, personal observation). A paucity of pollinators at WH may have led individuals in larger populations to compete more intensely than individuals in smaller populations for those pollinators that did arrive. Indeed, other studies have shown that intraspecific interactions among plants can become competitive when pollinators are scarce relative to population size. For example, Zimmerman (1980) demonstrated that seed set of *Polemonium foliosissimum* individuals declined as the ratio of bees to flowers declined in what he termed the “competitive phase” of the flowering season. Campbell and Husband (2007) came to a similar conclusion upon finding a negative relationship between pollinator visitation rate and population size in *Hymenoxys herbacea*. Moreover, at least seven other studies in a review on plant spacing and pollination (Ghazoul 2005) have found negative relationships between population size or patch size and either pollination or reproduction, suggesting that such relationships may be more than exceptions to the rule.

In a previous, observational study in natural populations of *S. angularis* (Spigler and Chang 2008), we similarly concluded that competition was important in affecting seed set. However, in that study, we suggested that competition for below-ground resources was responsible based on a negative relationship between seed set and local plant abundance. While we recognize that results from the current study should be interpreted with caution because of the low replication, together these two studies highlight the role of competition in influencing seed set in this species. By removing the role of below-ground resource competition, the current study adds to the previous study by further suggesting that competition for pollinators may influence the relationship between population size and seed set in *S. angularis*.

While a negative relationship between population size and seed set mediated through pollinators was initially unexpected, the nonlinear nature of this relationship is perhaps

less surprising in light of the biology of *S. angularis*. *Sabatia angularis* is capable of autonomous autogamy (R.B. Spigler, unpublished data), and this ability is thought to provide reproductive assurance in the face of pollen limitation (Karoly 1992; Kalisz et al. 2004). Accordingly, there may be a lower limit to which fruit and seed set decline in *S. angularis* in times of pollinator scarcity, assuming resources are not limiting, because at least some reproduction is possible through autonomous self-pollination. Complete reproductive assurance, however, is unlikely in *S. angularis*, because flowers are protandrous and autogamy is possible only when male and female phases overlap. Thus we might expect *S. angularis* individuals in natural populations to have at least some base level of reproductive assurance, provided there are enough resources, although pollination would be necessary for full seed set, leaving *S. angularis* susceptible to pollinator-mediated effects of population size.

It follows that such reproductive compensation via autogamy would also lead to an upper limit to the degree of pollen limitation experienced by *S. angularis* individuals. Although L , our measure of pollen limitation, was negative for the $N = 4$ population size class, indicating perhaps stigma clogging from the application of large quantities of pollen or mechanical damage to stigmas upon hand-pollination (Young and Young 1992), the amount of pollen limitation seen in the other two population size classes falls within the range of pollen limitation expected for a self-compatible, autogamous species (Larson and Barrett 2000). While we cannot make definitive conclusions about the shape of the relationship between population size and the degree of pollen limitation for seed set in this study because of low power, the data shown here are at least consistent with the potential for a limit to the degree of pollination limitation in this species and warrants further investigation.

In contrast with seed set, fruit set did not vary with population size across the experimental populations. Molano-Flores et al. (1999) suggested that fruit set might be decoupled from pollen receipt if a species requires only a minimal amount of pollen to set fruit. In general, this should be true for monocarpic species such as *S. angularis*, which are presumably under strong selective pressure to develop fruits. *Sabatia angularis* appears to require only a small amount of pollen for fruit set, as fruits can be formed with very few seeds (Dudash 1993; Spigler and Chang 2008). Consequently, we might expect seed set to be more responsive to changes in the pollination environment than fruit set, as seen in this study. Yet, Spigler and Chang (2008) found that fruit set can vary with population size in natural *S. angularis* populations, and in contrast with the pattern between seed set and population size seen in the current study, the former study demonstrated that fruit set is reduced in small populations. One possible explanation for the differences in results between the two studies is that effects of density on reproduction (e.g., Silander 1978; Allison 1990; Kunin 1992) would not be captured in the current study because it was held constant, but since population density does not influence mean fruit set at the population level in natural *S. angularis* populations (Spigler and Chang 2008), this explanation seems unlikely. It is also possible that the size range in this study was not sufficient to capture its relationship with fruit set. Given that the relationship observed in

natural populations was across a range of population sizes similar to that used in the current study (Spigler and Chang 2008), this explanation also seems unlikely to explain the discrepancy between the results. Finally, the difference in the effect of population size on fruit set between this experimental study and the earlier study using natural populations may be due to genetic causes. We did not attempt to identify genetic effects in the current study, but reduced genetic diversity and increased genetic load in small populations (Frankel and Soulé 1981; Barrett and Kohn 1991; Young et al. 1996) can often translate into reduced reproduction (Reed and Frankham 2003; Leimu et al. 2006). Genetic studies on natural populations of different sizes can test this possibility.

One caveat is that the unintended effects of the fungal pathogen *Cercospora sabbatae* found in the WH arrays may have influenced the results. *Cercospora sabbatae* is commonly found in natural *S. angularis* populations and can impact seed production in *S. angularis* individuals (R.B. Spigler, unpublished data). Nonetheless, a significant relationship between population size and seed set remained when the effect of this fungal pathogen was accounted for. Furthermore, results from the pollen supplementation experiment among healthy plants are consistent with increasing pollen limitation for seed set with population size at WH.

In conclusion, the results of this study suggest that large populations of *S. angularis* could face increased pollen limitation and decreased reproduction, potentially due to increased intraspecific competition for pollinators. Whereas facilitative effects in large populations are becoming more widely recognized as the need to protect rare and small plant populations increases, evidence from this study as well as others suggests that in some contexts, there are reproductive advantages in small populations. However, the benefits of small population size are likely to vary with a number of factors including the relative frequency of co-flowering species (Levin and Anderson 1970; Rathcke 1983) and their relative rewards to pollinators (Thomson and Plowright 1980; Stucky 1984; Kwak and Jennersten 1991). Moreover, these advantages may be tempered by genetic effects, which could influence the quality of offspring. Thus, future studies should consider such factors to improve our understanding of the relationships between population size and reproduction and to better predict the consequences for small populations.

Acknowledgements

This research was supported in part by funding awarded to R.B.S. from the Department of Plant Biology at the University of Georgia; Dean Grasso and the University of Georgia Graduate School; and the Georgia Native Plant Society. R.B.S. was also supported in part by a National Science Foundation Graduate Research Fellowship. The authors thank M. Boyd, A. Tull, J. Dadisman, N. Irwin, and W. Trask for assistance in the greenhouse and field. We also thank Whitehall Forest and D. Trapnell and M. Farmer for permission to work on their properties and J. Williams-Woodward for fungal pathogen identification. This manuscript was improved by comments from two anonymous reviewers.

References

Aizen, M.A., and Feinsinger, P. 1994. Forest fragmentation, polli-

- nation, and plant reproduction in a chaco dry forest, Argentina. *Ecology*, **75**(2): 330–351. doi:10.2307/1939538.
- Allee, W.C. 1931. Animal aggregations. A study in general sociology. University of Chicago Press, Chicago, Ill.
- Allee, W.C., Emerson, A.E., Park, O., Park, T., and Schmidt, K.P. 1949. Principles of animal ecology. Saunders, Philadelphia, Penn.
- Allison, T.D. 1990. Pollen production and plant density affect pollination and seed production in *Taxus canadensis*. *Ecology*, **71**(2): 516–522. doi:10.2307/1940305.
- Ashman, T.-L., Knight, T.M., Steets, J.A., Amaraskare, P., Burd, M., Campbell, D.R., Dudash, M.R., Johnston, M.O., Mazer, S.J., Mitchell, R.J., Morgan, M.T., and Wilson, W.G. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, **85**(9): 2408–2421. doi:10.1890/03-8024.
- Barrett, S.C.H., and Kohn, J.R. 1991. Genetic and evolutionary consequences of small population size in plant: implications for conservation. In *Genetics and conservation of rare plants. Edited by D.A. Falk and K.E. Holsinger*. Oxford University Press, New York, N.Y. pp. 3–30.
- Bell, J.M., Karron, J.D., and Mitchell, R.J. 2005. Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. *Ecology*, **86**(3): 762–771. doi:10.1890/04-0694.
- Bowers, M.A. 1985. Bumble bee colonization, extinction, and reproduction in subalpine meadows in Northeastern Utah. *Ecology*, **66**(3): 914–927. doi:10.2307/1940554.
- Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L.A., Kunstler, G., Liancourt, P., Tielbörger, K., Travis, J.M.J., Anthelme, F., Armas, C., Coll, L., Corcket, E., Delzon, S., Forey, E., Olofsson, Z.K.J., Pugnaire, F., Quiroz, C.L., Saccone, P., Schiffers, K., Touzard, M.S.B., and Michalet, R. 2008. Facilitation in plant communities: the past, the present, and the future. *J. Ecol.* **96**(1): 18–34.
- Brown, B.J., Mitchell, R.J., and Graham, S.A. 2002. Competition for pollination between an invasive species (purple loosestrife) and a native congener. *Ecology*, **83**(8): 2328–2336.
- Callaway, R.M. 1995. Positive interactions among plants. *Bot. Rev.* **61**(4): 306–349. doi:10.1007/BF02912621.
- Campbell, L.G., and Husband, B.C. 2007. Small populations are mate-poor but pollinator-rich in a rare, self-incompatible plant, *Hymenoxys herbacea* (Asteraceae). *New Phytol.* **174**: 915–925. doi:10.1111/j.1469-8137.2007.02045.x. PMID:17504472.
- Campbell, D.R., and Motten, A.F. 1985. The mechanism of competition for pollination between two forest herbs. *Ecology*, **66**(2): 554–563. doi:10.2307/1940404.
- Caruso, C.M. 1999. Pollination of *Ipomopsis aggregata* (Polemoniaceae): Effects of intra- vs. interspecific competition. *Am. J. Bot.* **86**(5): 663–668. doi:10.2307/2656575. PMID:10330069.
- Dudash, M.R. 1987. The reproductive biology of *Sabatia angularis* L. (Gentianaceae). Ph.D. thesis, Department of Biological Sciences, University of Illinois at Chicago, Chicago, Ill.
- Dudash, M.R. 1993. Variation in pollen limitation among individuals of *Sabatia angularis* (Gentianaceae). *Ecology*, **74**(3): 959–962. doi:10.2307/1940820.
- Frankel, O.H., and Soulé, M.E. 1981. Conservation and evolution. Cambridge University Press, Cambridge, UK.
- Galen, C., and Gregory, T. 1989. Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. *Oecologia (Berl.)*, **81**(1): 120–123. doi:10.1007/BF00377020.
- Ghazoul, J. 2005. Pollen and seed dispersal among dispersed plants. *Biol. Rev. Camb. Philos. Soc.* **80**(3): 413–443. doi:10.1017/S1464793105006731. PMID:16094807.

- Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. *J. Ecol.* **94**(2): 295–304. doi:10.1111/j.1365-2745.2006.01098.x.
- Groom, M.J. 1998. Allee effects limit population viability of an annual plant. *Am. Nat.* **151**(6): 487–496. doi:10.1086/286135. PMID:18811371.
- Hackney, E.E., and McGraw, J.B. 2001. Experimental demonstration of an Allee effect in American ginseng. *Conserv. Biol.* **15**(1): 129–136. doi:10.1046/j.1523-1739.2001.98546.x.
- Haig, D., and Westoby, M. 1988. On limits to seed production. *Am. Nat.* **131**(5): 757–759. doi:10.1086/284817.
- Harper, J.L. 1977. Population biology of plants. Academic Press, New York, N.Y.
- Kalisz, S., Vogler, D.M., and Hanley, K.M. 2004. Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature (London)*, **430**: 884–887. doi:10.1038/nature02776. PMID:15318220.
- Karoly, K. 1992. Pollinator limitation in the facultatively autogamous annual, *Lupinus nanus* (Leguminosae). *Am. J. Bot.* **79**(1): 49–56. doi:10.2307/2445196.
- Kéry, M., Matthies, D., and Spillman, H.H. 2000. Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. *J. Ecol.* **88**(1): 17–30. doi:10.1046/j.1365-2745.2000.00422.x.
- Knight, T.M. 2003. Floral density, pollen limitation, and reproductive success in *Trillium grandiflorum*. *Oecologia (Berl.)*, **137**(4): 557–563. doi:10.1007/s00442-003-1371-8.
- Kunin, W.E. 1992. Density and reproductive success in wild populations of *Diplotaxis erucoides* (Brassicaceae). *Oecologia (Berl.)*, **91**(1): 129–133.
- Kvålseth, T.O. 1985. Cautionary note about R^2 . *Am. Stat.* **39**(4): 279–285. doi:10.2307/2683704.
- Kwak, M.M., and Jennersten, O. 1991. Bumblebee visitation and seedset in *Melampyrum pratense* and *Viscaria vulgaris*: heterospecific pollen and pollen limitation. *Oecologia (Berl.)*, **86**(1): 99–104. doi:10.1007/BF00317395.
- Lamont, B.B., Klinkhamer, P.G.L., and Witkowski, E.T.F. 1993. Population fragmentation may reduce fertility to zero in *Banksia goodii* — a demonstration of the Allee effect. *Oecologia (Berl.)*, **94**: 446–450. doi:10.1007/BF00317122.
- Larson, B.M.H., and Barrett, S.C.H. 2000. A comparative analysis of pollen limitation in flowering plants. *Biol. J. Linn. Soc.* **69**: 503–520. doi:10.1111/j.1095-8312.2000.tb01221.x.
- Leimu, R., Mutikainen, P., Koricheva, J., and Fischer, M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? *J. Ecol.* **94**(5): 942–952. doi:10.1111/j.1365-2745.2006.01150.x.
- Levin, D.A., and Anderson, W.W. 1970. Competition for pollinators between simultaneously flowering species. *Am. Nat.* **104**(939): 455–467. doi:10.1086/282680.
- Mitchell, R.J. 1997. Effects of pollination intensity on *Lesquerella fendleri* seed set: variation among plants. *Oecologia (Berl.)*, **109**: 382–388. doi:10.1007/s004420050097.
- Moeller, D.A. 2004. Facilitative interactions among plants via shared pollinators. *Ecology*, **85**(12): 3289–3301. doi:10.1890/03-0810.
- Moeller, D.A. 2005. Pollinator community structure and sources of spatial variation in plant–pollinator interactions in *Clarkia xantiana* ssp. *xantiana*. *Oecologia (Berl.)*, **142**(1): 28–37. doi:10.1007/s00442-004-1693-1.
- Molano-Flores, B., Hendrix, S.D., and Heard, S.B. 1999. The effect of population size on stigma load, fruit set, and seed set in *Altilium stellatum* Ker. (Liliaceae). *Int. J. Plant Sci.* **160**(4): 753–757. doi:10.1086/314160.
- Mustajärvi, K., Siikamäki, P., Rytönen, S., and Lammi, A. 2001. Consequences of plant population size and density for plant–pollinator interactions and plant performance. *J. Ecol.* **89**(1): 80–87. doi:10.1046/j.1365-2745.2001.00521.x.
- Potts, S.G., Vulliamy, B., Roberts, S., O’Toole, C., Dafni, A., Ne’eman, G., and Willmer, P. 2004. Nectar resource diversity organises flower–visitor community structure. *Entomol. Exp. Appl.* **113**(2): 103–107. doi:10.1111/j.0013-8703.2004.00212.x.
- Rathcke, B. 1983. Competition and facilitation among plants for pollination. In *Pollination biology*. Edited by L. Real. Academic Press, Inc., Orlando, Fla. pp. 305–329.
- Reed, D.H., and Frankham, R. 2003. Correlation between fitness and genetic diversity. *Conserv. Biol.* **17**(1): 230–237. doi:10.1046/j.1523-1739.2003.01236.x.
- SAS Institute Inc. 1999. SAS user’s guide: statistics. SAS Institute Inc. Cary, N.C.
- Sih, A., and Baltus, M.-S. 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. *Ecology*, **68**(6): 1679–1690. doi:10.2307/1939860.
- Silander, J.A., Jr. 1978. Density-dependent control of reproductive success in *Cassia biflora*. *Biotropica*, **10**(4): 292–296. doi:10.2307/2387681.
- Spigler, R.B. 2007. The reproductive consequences of reduced population size in the biennial *Sabatia angularis* (Gentianaceae). Ph.D. thesis, Department of Plant Biology, University of Georgia, Athens, Ga.
- Spigler, R.B., and Chang, S.-M. 2008. Effects of plant abundance on reproductive success in the biennial *Sabatia angularis* (Gentianaceae): spatial scale matters. *J. Ecol.* **96**(2): 323–333. doi:10.1111/j.1365-2745.2007.01335.x.
- Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annu. Rev. Ecol. Syst.* **12**: 253–279. doi:10.1146/annurev.es.12.110181.001345.
- Stephenson, A.G., and Winsor, J.A. 1986. *Lotus corniculatus* regulates offspring quality through selective fruit abortion. *Evolution*, **40**(3): 453–458. doi:10.2307/2408568.
- Stucky, J.M. 1984. Forager attraction by sympatric *Ipomoea hederacea* and *I. purpurea* (Convolvulaceae) and corresponding forager behavior and energetics. *Am. J. Bot.* **71**(9): 1237–1244. doi:10.2307/2443648.
- Thomson, J.D., and Plowright, R.C. 1980. Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Dierivilla lonicera*. *Oecologia (Berl.)*, **46**(1): 68–74. doi:10.1007/BF00346968.
- Waser, N.M. 1978a. Interspecific pollen transfer and competition between co-occurring plant species. *Oecologia (Berl.)*, **36**(2): 223–236. doi:10.1007/BF00349811.
- Waser, N.M. 1978b. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. *Ecology*, **59**(5): 934–944. doi:10.2307/1938545.
- Waser, N.M., and Price, M.V. 1991. Reproductive costs of self-pollination in *Ipomopsis aggregate* (Polemoniaceae): are ovules usurped? *Am. J. Bot.* **78**(8): 1036–1043. doi:10.2307/2444892.
- Waser, N.M., and Real, L.A. 1979. Effective mutualism between sequentially flowering plant species. *Nature (London)*, **281**: 670–672. doi:10.1038/281670a0.
- Widén, B. 1993. Demographic and genetic effects on reproduction as related to population size in a rare, perennial herb, *Senecio integrifolius* (Asteraceae). *Biol. J. Linn. Soc.* **50**: 179–195. doi:10.1111/j.1095-8312.1993.tb00925.x.
- Young, H.J., and Young, T.P. 1992. Alternative outcomes of natural and experimental high pollen loads. *Ecology*, **73**(2): 639–647. doi:10.2307/1940770.
- Young, A.G., Boyle, T., and Brown, T. 1996. The population genetic

- consequences of habitat fragmentation for plants. *Trends Ecol. Evol.* **11**(10): 413–418. doi:10.1016/0169-5347(96)10045-8.
- Zimmerman, M. 1980. Reproduction in *Polemonium*: competition for pollinators. *Ecology*, **61**(3): 497–501. doi:10.2307/1937414.
- Ågren, J. 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology*, **77**(6): 1779–1790. doi:10.2307/2265783.