THE REPRODUCTIVE CONSEQUENCES OF REDUCED POPULATION SIZE IN THE BIENNIAL *SABATIA ANGULARIS* (GENTIANACEAE)

by

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(Under the Direction of Shu-Mei Chang and Stephen P. Hubbell)

ABSTRACT

Efforts to understand the factors affecting population persistence are at the forefront of population biology. Accumulated evidence suggests that small plant populations suffer fitness disadvantages such as reduced reproduction and increased inbreeding that ultimately might influence population viability. In this dissertation, I sought to improve our understanding of these processes by investigating the reproductive consequences of reduced population size in the widespread, native biennial *Sabatia angularis* (Gentianaceae).

First, I examined how fruit and seed set varied with population size, population density, and local neighborhood size, an individual-based index of conspecific density, across 20 natural *S. angularis* populations. I found that small populations have significantly reduced mean fruit set, perhaps due to reduced pollination in the smallest populations. Mean seed set per population did not vary with population size or density at the population level, and this result may be a net effect of significant, opposing facilitative and competitive interactions occurring on local spatial scales. These effects presumably weakened the effect of population size on subsequent reproductive success.

Second, I examined how genetic diversity and the mating system, characterized by the outcrossing rate, biparental inbreeding rate, effective number of male parents, and inbreeding coefficient, vary with population size and density. I demonstrate that outcrossing rate is significantly positively correlated with population size. Small populations had selfing rates as high as 40% compared to approximately 7% in the largest. Genetic diversity was unrelated to population size and density, and evidence suggests that selection for outcrossed offspring maintains levels of heterozygosity in small populations.

Lastly, I examined the presence and magnitude of inbreeding depression (ID) in *S. angularis* in two environments to understand the potential impact of increased selfing on population fitness. I demonstrate significant ID for germination and survival, which culminated in significant ID for a measure of cumulative fitness in both environments. Taken together, these studies suggest that small populations of *S. angularis* suffer significant reductions in fruit set and outcrossing rate, which might impact population fitness. These consequences are important as they have the potential to affect population viability and fundamental population processes such as colonization and local extinction.

INDEX WORDS: Inbreeding depression, fruit set, seed set, reproductive success, mating system, genetic diversity, population size, density, local neighborhood size, pollen load, *Sabatia angularis*

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In memory of my grandfathers, Leon Spigler and Morton Cohen.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Background

Ecologists have focused a disproportionate amount of attention on abundant species and the role of competition for resources in determining the outcome of ecological interactions. This has led to the paradigm that individuals in small populations should experience per capita benefits compared to individuals in large populations because of reductions in the severity of competitive interactions. What has been largely overlooked is that individuals in small populations may have difficulties encountering suitable mates, leading to decreases in fecundity that might offset any benefits gained from reduced competition (Allee 1931; Allee *et al.* 1949). This phenomenon, known as the "Allee effect", was originally proposed for social animals that depend upon cooperative behavior for protection, foraging, or mating (Allee 1931; Allee *et al.* 1949). At reduced population sizes and densities, Allee hypothesized that those populations would experience a reduction in these "services", and subsequently experience lowered survival and growth rates.

Strong parallels are evident in plants that depend on the service of pollinators for successful reproduction; these plants may be said to "cooperate" in an effort to increase pollinator visitation, to maintain genetic diversity, and to avoid inbreeding depression. Once population sizes become too small, individuals could face difficulties in accessing genetically compatible mates, disruptions in pollination services, and subsequent reduced offspring fitness. Indeed, accumulated evidence from rare and endangered plant populations over the past decade reveals that individuals in small populations suffer significant reproductive disadvantages including reduced reproductive success, reduced genetic diversity, and concomitant decreases in population fitness (reviewed in Leimu *et al.* 2006). Results are consistent across studies despite the variety of breeding systems and reproductive characteristics which they include: partially and fully self-compatible, self-incompatible, autogamous, dioecious, and protandrous. Such consequences may have severe implications for population viability. As populations continue to become more fragmented, these concerns have come to the forefront of population biology, and efforts to improve our understanding of how reproductive dynamics change at small population sizes are warranted.

Pollination & Reproductive Success

Perhaps the primary consequence of reduced population size is reduced reproductive success due to changes in the quantity or quality of pollination services. In general, pollinators visit small populations less often than large populations (Levin & Anderson 1970; Sih & Baltus 1987; Kunin 1997; Waites & Ågren 2004). As a result, individuals in small populations may not receive enough pollen to set full fruit or seed. For example, Ågren (1996) experimentally demonstrated that reduced seed set in small *Lythrum salicaria* populations compared to large populations was due to greater pollen limitation. Reduced pollinator visitation will be particularly detrimental to species that require a critical amount of pollen to set fruit (Campbell 1986; Mitchell 1997). However, reproductive losses via pollinator limitation are mitigated in species that require only a small amount of pollen (Molano-Flores *et al.* 1999) and in species capable of autogamy (self-pollination in the absence of pollinators) (Karoly 1992; Kalisz *et al.* 2004). Some autogamous species could nonetheless face reduced fruit and seed set (Stephenson 1981; Stephenson & Winsor 1986) or reduced progeny quality via inbreeding depression, the reduction in fitness of inbred progeny compared to outcrossed progeny (Lande & Schemske 1985; Schemske & Lande 1985; Charlesworth & Charlesworth 1987), which is discussed below. Even still, both self-incompatible and self-compatible plants can benefit from increased pollen deposition, since plants with higher pollen loads can produce more seeds than those with low pollen loads (Palmer & Zimmerman 1994; Brown & Kephart 1999).

Often, plant populations are not isolated but instead are embedded in a matrix of concurrently flowering species. In such cases, less common species may suffer decreases in reproductive success because they cannot compete successfully with more common flowering species for pollinator attraction (Levin & Anderson 1970; Waser 1978; Rathcke 1983). Alternatively, common species can serve as mutualists, attracting pollinators to rarer species in the community (Rathcke 1983; Feinsinger 1987). However, even if the rare species enjoys increased pollinator visitation rates, individuals can still suffer reproductive disadvantages. In mixed species assemblages, pollinators often move between flowers of different species indiscriminately, and such movement promotes disproportionate heterospecific pollen transfer to rarer species (Levin & Anderson 1970; Antonovics & Levin 1980; Kunin 1993; Kunin & Iwasa 1996; Kunin 1997). Heterospecific pollen transfer can physically clog stigmas, disabling conspecific pollen tubes from reaching ovaries, and pollen wastage when pollen is deposited on a different species. Both heterospecific pollen transfer (Waser 1978; Galen & Gregory 1989; Kunin 1997; Groom 1998) and pollen wastage (Campbell & Motten 1985; Feinsinger & Teibout 1991) have been shown to decrease seed set.

Alternatively, reductions in reproductive success in small populations may be independent of plant-pollinator interactions. For plant species with specific incompatibility systems, successful reproduction depends on the diversity of compatible mating types or alleles. Yet because of the effects of genetic drift, small populations are more likely to lose alleles by chance. As a result, small populations may contain a limited number of genotypically suitable mates, limiting reproduction (Byers & Meagher 1992; Brys *et al.* 2004; Ishihama *et al.* 2006).

The effects of reduced fecundity can be non-trivial. Several empirical studies have demonstrated significant decreases in seed production in plant populations with concomitant decreases in population size (Lamont *et al.* 1993; Widén 1993; Kéry *et al.* 2000; Hackney & McGraw 2001), population density (Kunin 1993; Roll *et al.* 1997) or both (Kunin 1997; Colas *et al.* 2001). If this pattern persists, such that individuals at low density or population size chronically suffer reduced fecundity, there may exist a critical threshold level below which the population growth rate becomes negative, ultimately causing the population to decline to extinction (Courchamp *et al.* 1999; Stephens & Sutherland 1999; Dennis 2002). The strength of the link between changes in fecundity and population growth, however, will depend upon whether populations are seed limited and whether density dependent processes during seedling emergence and survival are stronger than seed limitation. Turnbull *et al.* (2000) report that approximately 50% of seed augmentation experiments show seed limitation. In addition, empirical studies have demonstrated that reduced fecundity can translate into reduced recruitment (Ackerman *et al.* 1996; Lennartsson 2002), suggesting that changes in reproductive success in small populations may influence population growth rates. However, other studies suggest that seed limitation may not be the most important factor limiting population growth (e.g. Gustafsson & Ehrlén 2003).

Mating System, Genetic Diversity, & Fitness

In addition to facing reductions in reproductive success, small populations likely face changes in the mating system and reduced genetic diversity. These changes might arise through changes in pollination services or, alternatively, simply by chance. As discussed, small populations often suffer reductions in pollinator visitation rates. Self-compatible species may be able to offset pollinator limitation in small populations via autogamy. However, while this capability can buffer reproductive losses, it inevitably increases the rate of inbreeding. The selfing rate may also increase at low population densities due to changes in pollinator behavior. At low density, pollinators are more likely to move among flowers within a plant rather than between plants, thereby increasing intra-plant pollen transfer (Heinrich 1979; Antonovics & Levin 1980; Ghazoul *et al.* 1998) and the selfing rate of self-compatible species. Inbreeding may also increase through other avenues when population size or density declines. For example, in small populations, individuals are more likely to be related to each other by chance. Consequently, even random mating in small populations can lead to inbreeding events between related individuals, a phenomenon known as biparental inbreeding (Wright 1931).

Small populations also face the consequence of reduced genetic diversity (Frankel & Soulé 1981; Barrett & Kohn 1991; Young *et al.* 1996). Reduced genetic diversity in these populations arises through two main avenues: inbreeding and genetic drift (Ellstrand & Elam 1993). As discussed, small populations are more likely to experience greater rates of inbreeding than large populations. Given that inbreeding leads to a decrease in heterozygosity with each subsequent generation, small populations are expected to have lower genotypic diversity. Furthermore, although the effects of genetic drift are present in all populations, they are stronger in small populations. Alleles are expected to be fixed or lost in small populations at a greater rate than in large populations, causing reduced allelic richness in small populations (Wright 1931; Kimura & Crow 1964). Reductions in genetic diversity can significantly compromise population viability by potentially lowering population fitness. This suggestion comes from evidence demonstrating significant positive correlations between genetic diversity and various aspects of plant fitness (e.g., Bush *et al*, 1987; Oostermeijer *et al.* 1995; Fischer & Matthies

1998b; Paschke *et al.* 2002; Pluess & Stöcklin 2004). For example, Paschke *et al.* (2002) found fruit set and seed set increased with heterozygosity in *Cochlearia bavarica*, and Pluess and Stöcklin (2004) found a similar positive correlation between allelic diversity and mean biomass in *Scabiosa columbaria*.

Another consequence of inbreeding is the expression of inbreeding depression, the reduction in fitness of inbred progeny compared to outcrossed progeny (Lande & Schemske 1985; Schemske & Lande 1985; Charlesworth & Charlesworth 1987). These reductions can be severe. In fact, the fitness consequences of inbreeding can be so severe that inbreeding depression is considered the primary selective force maintaining the diversity of plant breeding systems (Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Uyenoyama *et al.* 1993; Barrett & Harder 1996). However, the same reductions in growth, survival, and reproduction caused by inbreeding depression could also impact population fitness and thus, population viability. Past studies, predominantly on commercial tree species, have attempted to quantify these effects by estimating genetic load or lethal equivalents in populations (e.g., Sorensen 1969; Franklin 1972). Recent studies have attempted to examine the population-level consequences of inbreeding depression more explicitly by incorporating inbreeding depression into demographic models (Mills & Smouse 1994; Frankham 1995; Oostermeijer 2000; Brook *et al.* 2002). These studies demonstrate that inbreeding depression can lower population growth rates and increase extinction risks. Such effects are believed to be more severe than reductions in reproductive success, but when combined, the consequences for population growth and persistence can be significant (Oostermeijer 2000).

Some uncertainty exists over the potential for inbreeding depression to affect population viability. Accumulating evidence illustrating variation in the presence and magnitude of inbreeding depression among maternal lines (e.g. Dudash *et al.* 1997; Mutikainen & Delph 1998; Píco *et al.* 2004) and environments (e.g. Dudash 1990; Ramsey & Vaughton 1998; Hayes *et al.* 2005) leads to the suggestion that inbreeding depression may not be sufficiently consistent or strong within a species to affect population dynamics. In addition, some suggest that the long term effects of inbreeding depression at the population level will be minimized through the purging of deleterious alleles exposed through inbreeding (Lande & Schemske 1985; Lande 1988). Reviews on the subject of purging reveal mixed evidence, but its effect has been demonstrated in several cases (Byers & Waller 1999; Crnokrak & Barrett 2002). Yet another view is that inbreeding is unlikely to drive populations to extinction before genetic factors take effect (Lande 1988; Pimm *et al.* 1989). This view, however, is beginning to shift.

The majority of studies examining the effect of population size on fecundity, genetic diversity, and fitness have heretofore focused on rare and endangered species and those recently affected by habitat fragmentation, with good reason. The implications of the reproductive consequences of reduced population size, however, apply not only to the conservation of rare and endangered species but also to basic population processes, such as colonization and local extinction. Despite this notion, the consequences of reduced population size are less studied in natural populations of common species. Thus, studies examining the reproductive consequences of reduced population size in common species will help to improve our understanding of the factors affecting fundamental population processes.

Dissertation Goals

In this dissertation, I examine the reproductive consequences of reduced population size in a native, widespread, relatively common biennial *Sabatia angularis* (L.) Pursh (Gentianaceae).

In the Chapter 2, I examine how reproductive success varies with population size and density across 20 natural *S. angularis* populations. I examine whether populations of this species are pollen limited and whether pollen loads increase with either population size or density. In addition to population level measures of abundance, I address these questions on local spatial scales. In Chapter 3, I examine how the mating system and genetic diversity vary with population size and density in nine natural *S. angularis* populations, which represent a subset of the original 20 populations. Using allozymes, I characterize the mating system by the level of outcrossing, biparental inbreeding, effective number of pollen donors, and the inbreeding coefficient of the parental generation. For each population, I estimate genetic diversity as the number of alleles per locus, the effective number of alleles per locus, and observed and expected heterozygosity. In Chapter 4, I address the potential demographic consequences of changes in the mating system by examining inbreeding depression in six natural *S. angularis* populations, which represent a subset of the nine populations from the previous study. I assess inbreeding depression in two environments: a common garden and the natural, maternal environment. I conclude by synthesizing the results from these chapters to evaluate the reproductive consequences of reduced population size in *S. angularis* and how these consequences might influence population viability.

This work represents a significant contribution to our understanding of how population dynamics can change at small population size. I determined that small populations of *S. angularis* have significantly reduced fruit set and significantly increased selfing. Furthermore, I demonstrated that inbreeding depression is prevalent in this species, with self progeny exhibiting significantly lower germination and survival than outcrossed progeny. Taken together, these results strongly suggest that individuals in small populations of *S. angularis* suffer reproductive disadvantages and that these disadvantages may be large enough to affect population level dynamics. Results from this study agree with previous studies on rare and endangered species and support the assertion that these consequences of reduced population size represent general phenomena. This work also demonstrates the utility of common species for investigating processes that impact the risk of extinction in rare species, and thus opens the door to more experimental investigations of these processes.

CHAPTER 2

EFFECTS OF PLANT ABUNDANCE ON REPRODUCTIVE SUCCESS IN THE WIDESPREAD, NATIVE BIENNIAL *SABATIA ANGULARIS* (GENTIANACEAE): SPATIAL SCALE MATTERS[1]

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Abstract[2]

1. Small and low-density populations often suffer significant reductions in reproduction, as exemplified by studies on rare and threatened species. This phenomenon is less studied in common species, but if general, should not be restricted to those in peril. We addressed this assertion by examining the effects of population size, population density, and local neighborhood size on fruit set, seed set, and subsequent reproductive success across 20 natural populations of the widespread biennial *Sabatia angularis*. We also examined pollen loads across a subset of populations to address whether changes in reproduction were a function of reduced pollination.
2. Populations ranged in size from one to an estimated 7,700 individuals and in density from 0.12 to 3.11 individuals/m^2. Population density had no effect on pollen load, fruit set, seed set, or reproductive success. Population size significantly affected fruit set across the study populations. Interestingly, the relationship between population size and fruit set was non-linear. Both small and large *S. angularis* populations had reduced mean fruit set relative to intermediate-sized populations. Although we did not find a significant relationship between population size and mean stigmatic pollen load, reduced pollen load in the smallest population suggests that reduced fruit set in the smallest populations may be due to pollen limitation. A similar quadratic pattern was seen between reproductive success and population size across all but the very largest population.
3. Local neighborhood size at 1m and 4m from focal study plants had significant but opposing effects on seed set and subsequent reproductive success. Although increases in the number of neighboring conspecifics within 1m reduced seed set and reproductive success, increases beyond that distance within 4m actually increased these variables. We

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[2] In *Journal of Ecology* format.

conclude that these effects are due to local competition at the smallest spatial scales and facilitation for pollination beyond that distance.

4. Our study confirms that reduced reproductive success in small plant populations can occur in common species as well as in rare and endangered species and supports the emerging viewpoint that increased reproductive success with population size is a general phenomenon. In contrast to most studies, however, we also found that further increases in population size beyond some point lead to reduced plant reproduction.
5. Lastly, this study highlights the complexity of ecological interactions affecting reproduction and the importance of incorporating multiple spatial scales when examining population dynamics.

Introduction

Evidence accumulating over the past decade demonstrates that plants in small and/or low-density populations often suffer decreases in reproductive success (hereafter, "RS") (e.g., Lamont *et al.* 1993; Widén 1993; Groom 1998; Colas *et al.* 2001; Hackney & McGraw 2001). For plants that rely on animal pollinators, such decreases in RS most often arise because of decreased pollination quantity or quality when plant abundance is low. The quantity of pollen received may decline because pollinators are less attracted to small flowering patches (Sih & Baltus 1987; Kunin 1997) or because individuals in small or low-density populations can not successfully compete for pollinators against more common flowering species (Levin & Anderson 1970; Waser 1978; Rathcke 1983). Consequently, reproduction becomes pollen limited in these populations, and fruit and seed set are reduced (Ågren 1996; Knight 2003). This effect can be mitigated in species that require only a small amount of pollen to set fruit or seed (Molano-Flores *et al.* 1999) or in those that can compensate via autogamy (self-pollination in the absence of pollinators) (Karoly 1992). Despite alleviating pollen limitation when pollinators are scarce, however, autogamy can still result in lower fruit set if inbred fruits are selectively aborted (Stephenson 1981; Stephenson & Winsor 1986) and can compromise offspring quality via inbreeding depression (Lande & Schemske 1985; Schemske & Lande 1985; Charlesworth & Charlesworth 1987). These effects render the RS of some self-compatible species just as vulnerable to decreases in population size as self-incompatible species (Aizen & Feinsinger 1994; Aizen *et al.* 2002).

In addition to decreased pollen receipt, pollen quality can become compromised in small and low density populations, affecting RS. Often this is mediated through changes in pollinator behavior. Pollinators commonly increase intra-plant movements relative to inter-plant movements when plants are at low density (Antonovics & Levin 1980; van Treuren *et al.* 1993a; Karron *et al.* 1995; Ghazoul *et al.* 1998). In self-incompatible plants, intra-plant pollinator movements decrease compatible pollen receipt, which can translate into decreased RS (Waites & Ågren 2004). In self-compatible plants, these pollinator movements result in greater self-fertilization rates, also potentially reducing offspring quantity or quality. Pollinators can cause further decreases in RS when they move readily between species, and this behavior is more likely when a given species is at low density (Levin & Anderson 1970; Antonovics & Levin 1980; Kunin 1993; Kunin & Iwasa 1996; Kunin 1997). Such inconstant movement leads to increased heterospecific pollen transfer, which can then cause decreased reproduction (Waser 1978; Campbell & Motten 1985; Galen & Gregory 1989). Even when pollinator activity is unaltered, the quality of pollen received and subsequent RS can decline in small populations of species with specific self-incompatibility systems. Small population size for these species often translates into a limited number of suitable mates and an increase in the probability of receiving incompatible pollen (Byers & Meagher 1992; Byers 1995; Brys *et al.* 2004; Ishihama *et al.* 2006).

The numerous reproductive disadvantages to individuals in small populations suggest that individuals in the largest populations should have the highest RS. Despite this logic, reproductive costs in large populations can also exist. For example, facilitative interactions between plants for pollinator visitation at low abundance can become competitive when abundance is high (Rathcke 1983). In such cases, per capita pollinator visitation rates actually decline in large or dense populations as plants compete for pollinators. Competition for resources can also become intense as per capita resource availability declines with increasing plant abundance (Harper 1977; Weiner 1982). This can result in decreased RS for plants at high abundance, regardless of pollinator visitation rates (Mustajärvi *et al.* 2001).

The mounting evidence of a positive effect of population size or density on reproduction in the case of rare, endangered, and threatened plants and those affected by habitat fragmentation has led to questions about the extent to which this is a general phenomenon for plant species (Leimu *et al.* 2006). Relatively few studies have examined whether this relationship holds for natural populations of more common species. Yet all species, even widespread and common species, have populations that vary naturally in size or density, and the reproductive consequences of reduced population size need not be restricted to rare or endangered species (Dennis 1989; Courchamp *et al.* 1999; Fowler & Ruxton 2002). As such, understanding the consequences of reduced population size may be vital to understanding fundamental population processes. However, evidence for a general pattern in widespread, common species is equivocal

(Platt *et al.* 1974; Roll *et al.* 1997; Molano-Flores & Hendrix 1999; Bosch & Waser 2001; Leimu & Syrjanen 2002; Kolb & Lindhorst 2006).

Furthermore, the majority of studies examining the potential consequences of decreased population size and density for RS have been conducted solely on population-level spatial scales, and often, only crude estimates of density are reported. Yet plants rarely are distributed uniformly within populations. As a result, measures of abundance on large scales often do not accurately represent crowding in a plant's local neighborhood. The distinction between crude density and crowding in a local neighborhood may be important since ecological interactions affecting RS may be described better on small spatial scales. For example, the effects of resource competition among individuals are often best characterized on local spatial scales because resources are typically localized and plants are sessile (Mack & Harper 1977; Pacala & Silander 1985; Silander & Pacala 1985). In addition, many pollinators are known to behave in ways that optimize foraging (Pyke *et al.* 1977; Pyke 1978), responding to local variation in density within a population (Silander 1978; Kunin 1993). However, because pollinator dynamics also operate on multiple spatial scales (Herrera 1988; Steffan-Dewenter *et al.* 2002), the best approach to understanding the response of RS to population size or density is to incorporate both population and local (individual-based) spatial scales. Although several studies have examined RS on local scales (Allison 1990; House 1992; Widén 1993; Roll *et al.* 1997; Ghazoul *et al.* 1998; Mitchell & Ankeny 2001), studies examining both are rare (but see Wagenius 2006; Gunton & Kunin 2007).

In this study, we evaluate the effect of plant abundance on RS in natural populations of the widespread, native biennial *Sabatia angularis* (L.) Pursh (Gentianaceae) on two spatial scales. We ask the following questions: (1) At the population level, do mean population fruit set, seed set, and subsequent reproductive success increase with population size and population density? (2) At the individual, or local, level, do per capita fruit set, seed set and reproductive success increase with local neighborhood size (LNS), defined as the number of neighboring conspecifics within 1- and 4-meter radii surrounding focal study plants? (3) How does the response of RS to plant abundance compare across spatial scales? We hypothesize that higher visitation rates in large or dense populations and pollinator facilitation in larger local neighborhoods will lead to increased reproduction. We test this hypothesis by examining (i) whether fruit and seed set increase with stigmatic pollen load, (ii) whether relationships exist between mean stigmatic pollen load and either population size or density, and (iii) whether individual stigmatic pollen load increases with LNS.

Materials and Methods

Study Species and Sites

Sabatia angularis is an herbaceous biennial native to the eastern United States and southeastern Canada. It is widely distributed throughout its range and may be found in a variety of habitats including glades, marshes, rocky outcrops, old fields, roadsides, prairies, and serpentine barrens (USGS 2003). *S. angularis* produces showy, pink flowers that are protandrous and self-compatible (Dudash 1987, 1990). In the study populations, most plants flower from July until late August, although some populations begin as early as mid to late June. Flowers last approximately four days (Dudash 1987), but can last over a week if unpollinated (Spigler, pers. obs.). Although flowers are protandrous, individuals produce flowers continuously during their flowering period, and consequently, display flowers in both sexual phases. A medium sized plant can present upwards of 50 open flowers at a time, and this overlap among flowers potentially facilitates geitonogamy (transfer of self pollen among flowers within a plant). Flowers are nectarless, and only offer pollen as a reward to a generalist suite of pollinators that include leaf-cutter bees (Megachilidae), sweat bees (Halictidae), andrenid bees (Andrenidae), small carpenter bees (Anthophoridae), and hover flies (Syrphidae) (Dudash 1987; Spigler, pers. obs.). Upon pollination, flowers develop into many seeded, dry, dehiscent capsules that remain attached to the parent plant and disperse seeds passively. Seeds germinate in spring and develop into rosettes by late fall. Rosettes overwinter until late in the following spring when plants begin to bolt.

From June-July 2004, we identified 20 *S. angularis* populations in Georgia, North Carolina, and South Carolina (Table 2.1). For each population, we searched to make sure there were no other *S. angularis* plants within at least a 100 m radius. The distance between populations within the same vicinity was typically greater than 500 m, and tens of kilometers separated most study populations. Because natural *S. angularis* populations are patchy and discrete, estimates of local population size are feasible and accurate. In all but the largest, we assessed population size by counting all reproductive individuals. Populations ranged in size from only one individual to an estimated 7,700 individuals (Table 2.1). The population size for the largest population, GP, represents an estimate since *S. angularis* plants at this site are spread throughout an approximately 1 ha prairie. Within the prairie, we randomly located a 300 m^2 plot in which to conduct the study, and this plot contained 231 individuals. We scaled this number to estimate the total population size in the prairie as approximately 7,700. Plant density appeared particularly uniform in this population, and we believe this estimate is adequate for our study.

We determined population-level density for each population by measuring the perimeter of the population, calculating its area, and dividing the population size by that area. Population density ranged from 0.12 to 3.11 individuals/m^2 among study populations and was independent of population size ($\rho_{17} = 0.42$, $p = 0.090$). Within each population, we tagged and mapped all individuals to the nearest centimeter. We used the map information to calculate the number of neighbors within 1, 2, and 4 m radii surrounding focal individuals (hereafter referred to as "local neighborhood size" or "LNS"). We included multiple radii since the effects of density can be scale dependent and may be strongest at small to intermediate spatial scales (Wagenius 2006). The ranges of average LNS for those study populations with more than two individuals were 0.57 – 19.5 (1m LNS), 1.37 – 44.5 (2m LNS), and 2.4 – 80.5 (4m LNS) (Table 2.1).

Estimating Fruit Set, Seed Set, & Reproductive Success

We recorded fruit and seed set for the 2004 flowering season. Beginning in July, we tagged up to 35 randomly chosen focal plants per study population and monitored them continuously until fruit maturation. Prior to flowering we counted the number of buds per focal plant and estimated per capita fruit set as the ratio of fruit number to total bud number. We collected up to 20 fruits per focal plant after ripening but prior to dehiscence to assess seed set. Seed set is typically defined as the number of seeds per fruit. However, seeds of this species are extremely small (approximately 0.025 ± 0.008 SD mg/seed) (Spigler, unpublished data), and counting seeds is impractical. Instead, we used an alternative method: we weighed all seeds per fruit en masse to the nearest 0.1 mg, and used this measurement as a proxy for seed set. This protocol is supported by a significant positive correlation between seed number and seed mass ($\rho_{11} = 0.88$, $p = 0.003$). Reproductive success is conventionally measured as the total number of seeds produced per plant. We calculated reproductive success by first converting average seed mass per fruit per plant into average seed number per fruit using a regression relating seed mass to seed weight (data not shown). We then multiplied average seed number per fruit by the total number of fruits produced by that individual to obtain an estimate of reproductive success.

Estimating Pollen Load

To determine whether pollen load is related to population- and individual-based measures of abundance, we estimated average stigmatic pollen load in the following eight populations that represent a range of population sizes and mean population densities: LI3, JRM, LI1, BC3, CFR, WF, DP, and WOM (in order of smallest to largest) (see Table 2.1). We collected approximately 10-15 stigmas over 5-7 days, depending on the population, during the flowering season from a random set of non-focal individuals. We used non-focal individuals to account for the possibility that stigma collection affected seed set. In population LI3, however, we had to collect stigmas from focal plants because the population consisted of only twelve plants. This protocol resulted in a smaller stigma sample size for population LI3 (n = 8). For those plants, we did not use fruits from which we collected stigmas to estimate per capita seed set. We collected stigmas using forceps, placed each in a glassine envelope, and stored them in an airtight container containing drierite in a cold room until processing.

To stain pollen grains for counting, we mounted stigmas on microscope slides in basic fuchsin jelly (Kearns & Inouye 1993). The extremely high pollen loads and small size of pollen grains rendered counting all grains on the entire stigma infeasible. Instead, we chose one lobe of the bilobed stigma randomly and counted pollen grains on the top quarter of the lobe. We captured digital images of the pollen-covered lobe under a dissecting microscope and counted grains in an expanded image. We estimated pollen load as the number of pollen grains per sampled stigma portion.

Statistical Analyses

Fruit Set, Seed Set, & Reproductive Success

To test the predictions that mean fruit set, seed set, and reproductive success (RS) increase with either population size or density, we first calculated population mean trait values for each response variable. We calculated mean population seed set as the average of mean seed set per focal plant over all individuals in a given population. We arcsine-square root transformed mean fruit set values and log transformed RS to conform to model assumptions of normality. Population BCE had extremely high fruit (0.97) and seed set (12.25 mg/fruit ± 3.94 SD) and was removed as a statistical outlier according to Cook's D statistic. Across the remaining populations, a scatter plot of mean fruit set against population size revealed a potentially quadratic relationship (Fig. 2.1). Therefore, we evaluated the effect of population size on mean fruit set using both linear and quadratic models and did the same for seed set and RS to be consistent. We only used linear regression models, however, to evaluate the effect of density on the response variables, since no quadratic relationships were apparent from scatter plots with density. To reduce any bias in the relationships caused by the extremely large size of population GP and comparatively large density of population WOM (see Table 2.1), we log transformed population size and density values. We included mean population plant height as a covariate in the reproductive success analyses. Plant height can influence the number of fruits per plant, which directly affects RS, and plant height can vary across populations independently of population size and density. Previous analyses showed that the study populations vary significantly in mean plant height ($F_1 = 11.96$, $p < 0.0001$) and that this relationship was not due to population size or density ($p > 0.05$) (Spigler, unpublished data). All models were run using Proc GLM in SAS (1999).

We analyzed the effect of local neighborhood size on individual fruit set, seed set, and RS using mixed multiple regression models. We included individual-based 1m and 4m LNS as fixed effects and population identity as a random effect to account for variation attributable to differences among populations. We removed 2m LNS estimates from the analyses due to high correlations with both 1m LNS ($r_{388} = 0.76$, $p < 0.0001$) and 4m LNS ($r_{388} = 0.830$, $p < 0.0001$). In all analyses including LNS, we treated 4m LNS as the number of *additional* individuals beyond the 1m LNS to increase the independence of these factors. Almost 30% of individuals across all populations had complete (100%) fruit set, and the data could not be normalized. For this reason, we treated fruit set as a probability following a binomial distribution and used a generalized linear mixed model (Proc GLIMMIX in SAS), which allows response variables to follow non-normal distributions in a mixed model framework (Littell *et al.* 1996). We used general linear mixed models (Proc MIXED in SAS) to examine the effect of 1m and 4m LNS on seed set (square root transformed) and RS (log transformed) and included population identity as a random effect. As before, we included plant height as a fixed covariate in the RS analysis. We log transformed 1m and 4m LNS to decrease the high skew of these variables. Interactions between fixed effects were also examined. Non-significant interaction terms were removed from the model, and the model was rerun to evaluate significance of remaining effects. For all mixed models, we used Satterthwaite's approximation to determine degrees of freedom for fixed effects. We used likelihood ratio tests with 1 d.f. to test the significance of the random effect, population identity.

We selected individual study plants randomly. However, underlying environmental variables may cause spatial autocorrelation that could influence the individual-based results. To account for this possibility, we initially incorporated the spatial data in the individual-based analyses using the exponential model for spatial correlation in the MIXED and GLIMMIX procedures in SAS (Littell *et al.* 1996). These procedures automatically adjust fixed-effect estimates for spatial correlation when spatial data are included as random variables. The significance of spatial autocorrelation can be evaluated with a likelihood ratio test with 1 d.f. when comparing models including and excluding the spatial information. Preliminary results confirmed that spatial autocorrelation among focal plants in this study was not significant ($p > 0.05$), and therefore we present results from analyses excluding this additional factor below.

Pollen load

We used regression analysis to evaluate the effect of population size and density on mean pollen load per population. We did not include a quadratic model since there was no indication of a quadratic relationship from the raw data. To examine if positive relationships also occurred on an individual-based scale with respect to local neighborhood size, we used a mixed model approach. We included individual stigmatic pollen load as the dependent variable, 1m and 4m LNS (log transformed) as fixed effects, and population ID as a random effect. For the mixed model analysis, we square root transformed individual pollen load to normalize the data and to conform to model assumptions.

We also assessed whether differences in pollen load translate into differential fruit or seed set at the population level. We used regression analysis to examine the effect of mean stigmatic pollen load per population on mean population fruit and seed set. We could not address these at the individual level since we collected stigmas from predominantly non-focal plants for which we do not have fruit and seed set data.

Results

Fruit Set

At the population level, there was a significant quadratic relationship between population size and mean population fruit set (Fig. 2.1; Table 2.2). This model explained 49% of the variation in fruit set among populations. A linear relationship was not significant (Fig. 2.1; Table 2.2). Because we did not find populations with a number of individuals between 355 (WOM) and 7,700 (GP) individuals, it is unclear whether fruit set for GP is representative of all larger populations. Therefore, to corroborate the quadratic relationship among the remaining populations, we also analyzed the data without population GP. A log-transformation was not needed for population size in this analysis since population size was evenly distributed once GP was removed. Results from these analyses confirmed a significant quadratic relationship between population size and fruit set ($F_2 = 5.00$, $p = 0.023$, $R^2 = 0.42$) and similarly rejected a linear relationship ($F_1 = 2.28$, $p = 0.152$, $R^2 = 0.13$). In contrast, density was not related to fruit set (Fig. 2.1; Table 2.2). Overall, mean fruit set across populations was 0.75 (± 0.15 SD) and ranged from a mean population value of 0.45 in BCTM to 0.97 in BCE.

At the individual level, fruit set could not be explained by variation in local neighborhood size. Neither 1m nor 4m LNS significantly predicted fruit set, nor did their interaction (Table 2.3). Variation in fruit set among populations, however, was highly significant ($\chi^2_1 = 34.8$, $p < 0.0001$).

Seed set

Mean population seed set, measured as average total seed weight per fruit per population, could not be explained by a linear or quadratic relationship with either population size or density (Fig.

2.1, Table 2.2). Neither model changed qualitatively when population GP was removed from the dataset, further confirming any relationship is, at best, weak. Average seed set across populations was 7.72 mg (± 3.11 SD), which translates into approximately 309 seeds per fruit. However, the range was large. Mean seed set per population varied from 2.31 mg (± 1.85 SD) (approx. 92 seeds/fruit) in BCTE (2 plants) to 13.87 mg (± 5.10 SD) (approx. 555 seeds/fruit) in CFR, a mid-sized population (98 plants).

Individual-based analysis for the effect of local neighborhood size on per capita seed set across populations revealed that both 1m LNS and 4m LNS had significant, but opposing, effects on seed set. There was a significant, negative effect of 1m LNS on seed set (slope = -0.3066 with a 95% CI = -0.5812 to -0.0320), while 4m LNS had a significant, positive effect (slope = 0.3168 with a 95% CI = 0.0265 to 0.6070) (Table 2.3). The interaction between these two parameters was not significant ($p > 0.05$). Average seed set per plant ranged considerably across individuals, varying over nearly two orders of magnitude from only 0.90 mg (± 1.56 SD) (approx. 36 seeds per fruit) for an individual in population CC to 25.05 mg (± 8.90 SD) (approx. 1002 seeds) for an individual in population CFR. In addition to LNS, population identity explained a significant portion of this variation in seed set ($\chi^2_1 = 109.0$, $p < 0.0001$).

Reproductive Success

A linear relationship between mean population reproductive success and population size was positive, but this fell short of significance (Table 2.4). There was no evidence of a quadratic relationship over the entire range of population sizes examined (Table 2.4). This changed when population GP was removed from the dataset; a quadratic relationship significantly explained 86% of the variation in reproductive success across the remaining populations ($F_3 = 26.93$, p <

0.0001). Density did not significantly affect reproductive success. In all analyses, mean plant height explained a significant amount of the variation in reproductive success across populations, as expected (Table 2.4).

Local neighborhood size at both the 1m and 4m scales had significant effects on reproductive success (Table 2.4). Similar to results seen for seed set, the direction of these effects was scale-dependent. At the smallest scale, 1m LNS had a negative effect on reproductive success (slope = -0.164 with a 95% CI = -0.3267 to -0.0015), while the number of neighboring conspecifics beyond 1m and within 4m had a positive effect (slope = 0.215 with a 95% CI = 0.0534 to 0.3770). Average reproductive success per plant across populations was 9,173.6 (± 16, 522.84 SD). The plant with the greatest reproductive success came from population CFR. This plant produced almost 150,000 seeds over 147 fruits. The plant with the lowest reproductive success came from population BC2 and produced only about 22 seeds from one fruit. In addition to LNS, plant height (Table 2.4) and population identity explained significant variation in reproductive success ($\chi^2_1 = 41.4$, $p < 0.0001$).

Pollen load

At the population level, neither population size nor density explained significant variation in mean stigmatic pollen load per population (Fig. 2.2; Table 2.2). However, Tukey's HSD post hoc test showed that mean pollen load in the smallest population examined, population LI3 with only 12 individuals, was significantly lower than all other populations examined ($F_1 = 8.70$, $p = 0.004$). Across populations, mean pollen load was 405 (± 137 SD) grains. Population CFR received the most pollen grains per stigma sample (634 grains ± 194 SD), and population LI3 received the least amount (206 grains ± 98 SD).

At the individual level, neither the 1m or 4m LNS significantly predicted individual stigmatic pollen load (Table 2.3). However, there was a trend toward a positive effect of 4m LNS on pollen load, suggesting that larger neighborhood sizes at this spatial scale might increase pollen load (slope = 2.790 with a 95% CI = -0.1711 to 5.7508) (Table 2.3). Individuals varied greatly in pollen load from only 58 grains on a stigma sampled from a plant in population LI3 to 1,186 grains on a stigma sampled from a plant from population JRM. We rarely found heterospecific pollen grains on the collected stigmas, and therefore excluded its potential influence on reproductive success in these populations. Population identity accounted for significant variation in pollen load ($\chi^2_1 = 11.5$, $p < 0.001$).

A linear relationship between pollen load and fruit set was not significant at the population level ($F_1 = 3.16$, $p = 0.135$). Instead, mean fruit set values appeared to saturate as pollen load on the sampled stigmas reached about 450 grains or higher (Fig. 2.3). Across those populations with mean pollen loads less than this amount, a linear relationship was significant and explained 93% of the variation in fruit set ($F_1 = 39.19$, $p = 0.008$). There was a significant, positive relationship between mean population seed set and mean population pollen load over the entire range of pollen loads examined ($F_1 = 16.74$, $p = 0.006$) (Fig. 2.3). This linear relationship was strong and explained approx. 74% of the variation in seed set across populations.

Discussion

Population-level Effects

We predicted that mean population fruit set, seed set, and subsequent reproductive success (RS) would increase with either population size or density across populations of the widespread, native biennial *Sabatia angularis*. This prediction was met for population size and supports the emerging principle that reproduction is reduced in small plant populations (reviewed in Leimu *et al.* 2006). Mean fruit set responded significantly to variation in population size, and there was some suggestion that mean RS increases with population size as well.

A surprising result from this study was that large populations also showed reduced fruit set and may also exhibit reduced RS. The largest population, GP, ranked in the bottom five populations for fruit set and had the second lowest seed set. Most populations do not get as large as population GP, which had an estimated 7,700 individuals, and when this happens the data suggest there can be severe decreases in fruit set. Part of this decrease could be site specific since the prairie where these plants reside consists of calcareous soils that are low in organic matter and can be physically stressful on roots due to seasonal shrinking and swelling of the soil. However, even when population GP was removed from the analyses, the quadratic relationship between population size and mean fruit set persisted and a similar quadratic relationship between population size and mean reproductive success over the remaining populations became significant, suggesting that intermediate-sized populations of *S. angularis*, not the largest, enjoy the highest RS.

This hump-shaped pattern is a distinguishing feature of a phenomenon referred to as the Allee effect (Allee 1931; Allee *et al.* 1949; Odum & Allee 1954). The Allee effect recognizes that different intra-specific dynamics occurring in small and large populations can lead to decreased fitness in those populations. When populations are small, facilitative intra-specific interactions result in a positive relationship between some aspect of fitness and population size (Stephens *et al.* 1999). Above some population size, interactions among individuals cease to be positive, turning negative as population sizes increase further. As a result, intermediate-sized populations enjoy the greatest fitness. The negative effects of intra-specific interactions via competition in large populations are well documented within the plant competition literature (Harper 1977; Weiner 1982; Pacala & Silander 1985; Silander & Pacala 1985). However, empirical studies examining the positive effects of population size in plant populations are comparatively recent.

In this study, we show that there is a positive relationship between fruit set and the number of individuals across small populations. These reductions may be due to pollen limited fruit production in the smallest populations. Previous research has demonstrated pollen-limited fruit and seed set in natural *S. angularis* populations (Dudash 1993). Fruit set did increase sharply with pollen load up to approximately 450 grains, beyond which the data suggest fruit set begins to saturate with additional pollen receipt. Such saturation is an inevitable consequence of defining fruit set as a ratio that cannot increase above one. Maximum fruit set in the study populations, however, did not reach this high. Instead, the apparent saturation might suggest that factors other than pollen receipt limit reproduction beyond some point (Silander & Primack 1978; Bertin 1990; Mitchell 1997).

Despite the fact that fruit set in some of the study populations appear to be pollen limited, mean stigmatic pollen load did not increase with population size. This casts doubt on the role of pollen limitation underlying the relationship between mean fruit set and population size. We did find, however, that individuals in the smallest study population included in the pollen load study had significantly less pollen per stigma, on average, than individuals in the other seven populations combined. Rather than changing linearly with population size, it is possible that pollen load drops precipitously below a certain size. This effect was demonstrated most strikingly in a study on *Clarkia concinna* (Groom 1998) and might be responsible for decreased fruit set in small *S. angularis* populations. This hypothesis is also consistent with the observation that populations with fewer than 15 individuals appear to have lower mean seed set than larger populations. Furthermore, the lack of a continuous relationship between pollen load per flower and population size might also explain why seed set did not respond to increases in population size across the entire range of populations, given a strong and significant linear relationship with pollen load that suggested seed set was pollen limited.

Although the pollen data does suggest that pollen load per flower does not change over the majority of populations examined, it does not provide information about how many flowers per plant are visited. Yet if pollinators visited a lower proportion of flowers per plant in large populations, this might explain why fruit set, but not seed set, was reduced in those populations. In large populations, plants may receive fewer visits per plant, even if they attract a large number of pollinators. This can occur because pollinators depart sooner in large populations, consistent with optimal foraging theory (Goulson 2000), or because plants in these populations begin to compete for pollinator visitation when pollinator abundance is low relative to the number of plants (Rathcke 1983). Such differences in pollinator visitation to individual plants should result in greater variation in stigmatic pollen load in the largest populations. This possibility can be explored further by collecting more stigmas per plant or with pollinator observations. Unfortunately, initial attempts to watch pollinators in the study populations proved inefficient since pollinator visitation rates per hour were extremely low.

Alternative mechanisms to explain the reductions in fruit set in small and large *S. angularis* populations exist, but are less likely. *S. angularis* is self-compatible, and despite protandry, autogamy does occur (Spigler, unpublished data). Increased self-fertilization in response to reduced pollinator visitation could have caused the observed reduction in RS if inbred fruits were selectively aborted. However, neither fruit nor seed set appear to be affected by inbreeding depression in this species (Dudash 1990; Spigler, unpublished data). Alternatively, reduced fruit set in small populations may occur because those populations are located in poorer environments (Eisto *et al.* 2000; Vergeer *et al.* 2003). Such an association between habitat quality and population size can lead to erroneous conclusions about a causal relationship between reproduction and population size (Bosch & Waser 2001). If small populations of *S. angularis* were located in poor habitats, we might also expect those populations to have shorter plants or plants with fewer buds, on average. This was not the case ($r_{\text{height, 18}} = -0.048$, $p = 0.849$, $r_{\text{buds, 18}} = 0.042$, $p = 0.869$) (Spigler, unpublished data). Experimental manipulations of population size would help to elucidate the mechanism responsible for reduced fruit set in small populations of *S. angularis*.

Decreased resource availability due to increased competition, however, is the conventional interpretation for decreases in reproduction with increased plant abundance. For example, Mustajärvi *et al.* (2001) suggested increased competition in dense populations of *Lychnis viscaria* likely caused lowered capsule production in these plants relative to sparse populations. Yet in our study, population size, not density, predicted fruit set. Population size does not account for plant spacing, and as such is less likely to indicate the intensity of competition for resources. Reduced fruit set in large populations could be related to the effects of a fungal pathogen. *S. angularis* is the specific host for the pathogen *Cercospora sabbatiae*. This pathogen caused leaf necrosis and general wilting (Spigler, pers. obs.). While population size is not directly related to the frequency of this fungus, its interaction with habitat type influences the probability of fungal infection (Spigler, unpublished data).

Local-level Effects

Local neighborhood size explained significant variation in per capita seed set and reproductive success, but not fruit set, at both the 1m and 4m scale. These effects were surprisingly distinct. At the smallest spatial scale, increases in local neighborhood size within one meter of focal plants actually decreased seed set and subsequent RS. This linear decrease is highly suggestive of intra-specific competition for resources. This hypothesis is supported by a significant negative correlation between 1m LNS and total number of buds per plant ($r_{350} = -0.179$, $p < 0.001$), which in turn, is correlated with seed set ($r_{367} = 0.20$, $p < 0.0001$) (Spigler, unpublished data). In contrast, increasing neighborhood size at the 4m scale significantly increased seed set and RS. Roll *et al.* (1997) similarly found that increased neighborhood density facilitated reproduction in *Lesquerella fendleri* due to increased pollinator attraction. Although not significant, increases in the number of additional neighbors at the 4m spatial scale in this study tended to increase individual pollen load. Including a larger sample of complete stigmas from more populations in future studies would help clarify the relationship between LNS and pollen receipt.

These results stand in contrast to our initial predictions that the effect of local neighborhood size on reproduction would parallel those found at the population level. Whereas effects of plant abundance on fruit set were apparent only at the population level, seed set and subsequent reproductive success were only affected by plant abundance on local spatial scales. The opposing effects at the 1m and 4m spatial scales suggest that competition for resources in *S. angularis* occurs on very local scales and that both are important for predicting individual seed set and subsequent reproductive success within *S. angularis* populations. The negative effects of competition apparent at this spatial scale presumably did not prevail at the population level because crude estimates of density often do not accurately represent local competitive neighborhoods.

Two main caveats should be mentioned before reaching final conclusions from this study. First, this study was conducted over one flowering season. The pattern between population size and RS and that between local neighborhood size and RS could vary over years depending on pollinator abundance, weather, or resource availability (e.g. Molano-Flores & Hendrix 1999). Second, although population BCE, comprised of only one individual, was removed from the data set as a statistical outlier, its high RS nonetheless represents a biological reality and should not be ignored. It demonstrates that plants in small populations – in fact the smallest – need not always experience lowered reproduction.

Conclusions

This study reached several novel conclusions. First, it reveals a hump-shaped relationship between population size and reproduction, not a simple linear relationship. Small and large populations of *S. angularis* both suffered reproductive disadvantages, exhibiting the pattern expected with an Allee effect. Decreased pollinator visitation may explain these reductions in small populations, due to overall decreased visitation. In large populations, reduced mean fruit set and mean reproductive success may be due to intra-specific competition for pollinators, increased occurrence of fungal infection, or other ecological interactions or abiotic factors. Second, we found that the consequences of plant abundance for reproduction were scale-dependent. Wagenius (2006) also examined the effect of multiple local and population levels of abundance on reproduction in *Echinacea angustifolia*, but found reproduction increased with population size at all scales examined. In contrast, overall population size, 1m LNS, and 4m

LNS all had distinct effects on reproduction in *S. angularis*. These differences reflect differences in the mechanisms affecting reproduction at each spatial scale and highlight the complexity of ecological interactions.

More generally, this study adds to the growing body of evidence demonstrating reduced reproduction in small plant populations. The overwhelming majority of studies comprising this body so far has focused on rare or endangered species, leaving open the question of whether this phenomenon applies to the dynamics of undisturbed, natural populations of common species as well. We found that small populations of *S. angularis* experience decreased reproduction, despite being widespread and self-compatible. This result is consistent with other studies on much less common species, such as the rare plants *Gentianella germanica* (Fischer & Matthies 1998a) and *Primula vulgaris* (Brys *et al.* 2004) and the endangered plant *Rutidosis leptorrhynchoides* (Morgan 1999). These comparisons are important, because they suggest that common species are not impervious to the negative effects of decreased population size. As such, these findings may be relevant to our basic understanding of population dynamics.

Table 2.1. Summary data for study populations including location, population size, population density, and average local neighborhood size. †Population size of GP was estimated from a 300 m^2 subplot consisting of 231 reproductive adults within the approximately 1 ha prairie.

					Average Local Neighborhood Size (SD)	
Population	**Abbreviation**	**Location**	**Population Size**	**Raw Density**	**r = 1m**	**r = 4m**
Buck Creek Extension	BCE	Clay Co., NC	1	N/A	0	0
Buck Creek Trail (Middle)	BCTM	Clay Co., NC	1	N/A	0	0
Buck Creek Trail (End)	BCTE	Clay Co., NC	2	N/A	0	1
Lake Issaqueena 3	LI3	Pickens Co., SC	12	0.261	1.600 (0.993)	2.400 (2.534)
Highway 175	HWY	Clay Co., NC	14	1.198	7.200 (0.993)	8.000 (2.534)
Coweeta 2	COW2	Macon Co, NC	15	0.136	1.545 (0.946)	6.000 (2.416)
Hiawassee	HIA	Floyd Co., GA	36	0.136	1.714 (0.839)	10.643 (2.142)
Coweeta 1	COW1	Macon Co, NC	40	0.606	5.296 (0.604)	20.000 (1.542)
Jackrabbit Mountain	JRM	Clay Co., NC	55	0.797	3.182 (0.546)	10.212 (1.395)
Lake Issaqueena 1	LI1	Pickens Co., SC	57	0.142	0.571 (0.530)	5.057 (1.355)
Buck Creek 3	BC3	Clay Co., NC	60	0.166	3.210 (0.538)	5.412 (1.374)
Buck Creek 1	BC1	Clay Co., NC	66	0.122	0.867 (1.060)	9.133 (4.533)
Lake Issaqueena 2	LI2	Pickens Co., SC	76	0.247	1.071 (0.839)	8.786 (2.142)
Cedar Cliff	CC	Macon Co, NC	97	1.276	5.414 (0.583)	15.897 (1.489)
Caney Fork Rd.	CFR	Jackson Co., NC	98	0.356	2.296 (0.604)	10.630 (1.542)
Buck Creek 2	BC2	Clay Co., NC	141	0.606	8.800 (6.298)	30.040 (12.844)
Wykle Field	WF	Macon Co, NC	220	0.733	3.258 (0.564)	33.871 (1.439)
Dry Prairie	DP	Floyd Co., GA	239	0.797	3.750 (0.702)	44.350 (1.792)
West Old Murphy Rd.	WOM	Clay Co., NC	355	3.114	19.462 (9.149)	80.538 (29.193)
Grand Prairie	GP	Floyd Co., GA	7700†	0.770	3.00 (0.655)	34.652 (1.671)

Table 2.2. Effect of population size and population density on mean population fruit set, seed set, and pollen load. Population size and population density were log transformed. Fruit set was arcsine square-root transformed. Results from both the linear and quadratic regression models including population size are listed. R^2 values are adjusted for the quadratic models.

† Population WOM is not included in fruit set analyses because we did not have information on bud numbers per plant.

	df	F	p	Model R^2
A. Fruit Set†				
1. Population Size				
(a) Linear Model	1	3.36	0.086	0.17
Population Size	1	3.36	0.086	
(b) Quadratic Model	2	9.05	0.003	0.49
Population Size	1	17.33	0.001	
(Population Size)*(Population Size)	1	12.35	0.003	
2. Population Density	1	2.05	0.175	0.13
Population Density	1	2.05	0.175	
B. Seed Set				
1. Population Size				
(a) Linear Model	1	0.01	0.923	< 0.01
Population Size	1	0.01	0.923	
(b) Quadratic Model	2	2.10	0.155	0.11
Population Size	1	3.76	0.070	
(Population Size)*(Population Size)	1	4.19	0.058	
2. Population Density	1	0.27	0.611	0.02
Population Density	1	0.27	0.611	
C. Pollen Load				
Population Size	1	0.12	0.747	0.02
Population Density	1	0.31	0.595	0.05

Table 2.3. The effects of local neighborhood size (LNS) on individual fruit set, seed set, and pollen load. LNS within 4m represents the number of additional individuals between a 1m radius and 4m radius. LNS values were log transformed and seed set and pollen load were square root transformed. Individuals from WOM were not included in the fruit set analysis.

	df	**F**	**p**
Fruit Set			
1m LNS	1, 357	1.42	0.234
4m LNS	1, 168	2.63	0.107
Seed Set			
1m LNS	1, 382	4.82	0.029
4m LNS	1, 248	4.62	0.033
Pollen Load			
1m LNS	1, 92.2	1.33	0.256
4m LNS	1, 43.2	3.61	0.064

Table 2.4. The effects of (A) population size, (B) density, and (C) local neighborhood size on reproductive success. Results for linear and quadratic models are shown for population size. Analyses at the population level (A,B) are on mean population fitnes. Height was included as a covariate in all analyses. Population size, density, and local neighborhood size, and reproductive success were log transformed.

	df	**F**	**p**
A. Population Size			
1. Linear Model			
Population size	1	3.56	0.079
Plant height	1	48.13	< 0.0001
2. Quadratic Model			
Population size	1	0.36	0.556
Population size*Population size	1	0.44	0.516
Plant height	1	6.80	< 0.0001
B. Population Density			
Density	1	2.38	0.147
Plant height	1	26.12	< 0.001
C. Local Neighborhod Size			
1m LNS	1, 334	3.94	0.048
4m LNS	1, 150	6.90	0.010
Plant height	1, 317	202.70	< 0.0001

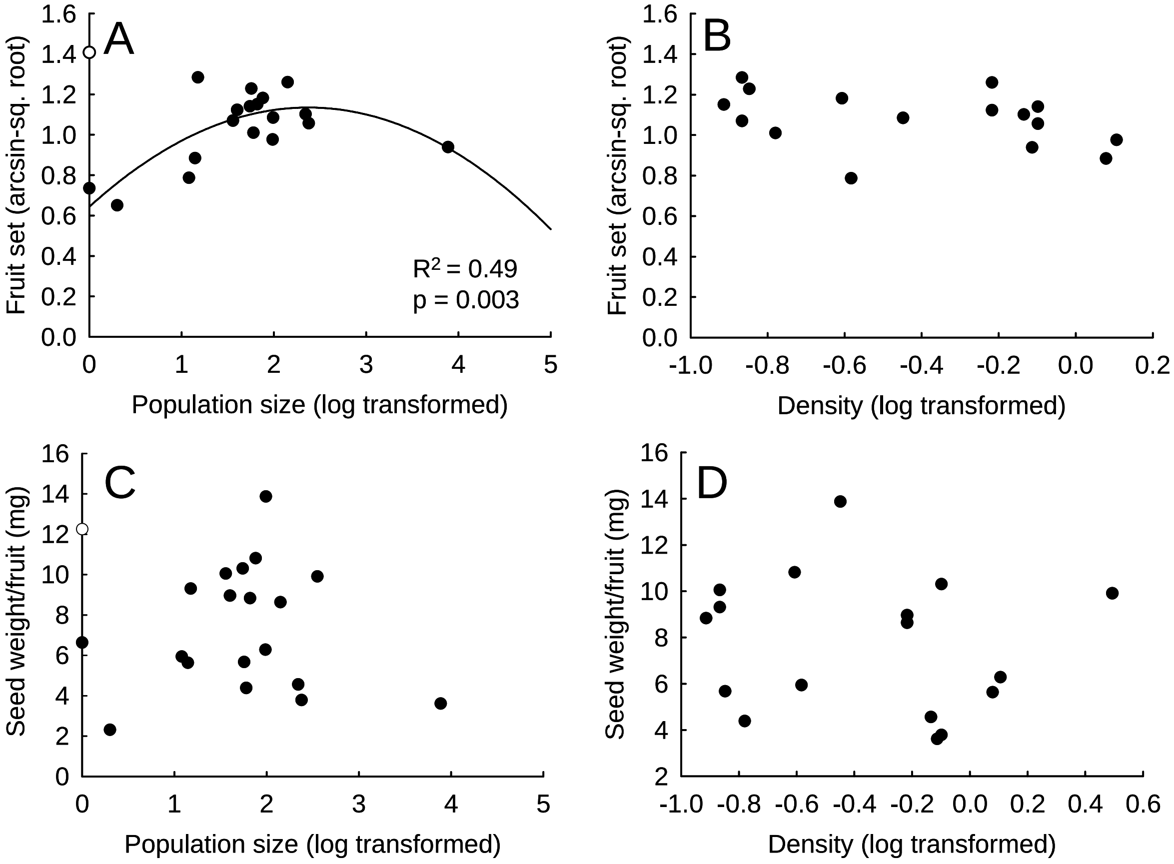

Figure 2.1. Relationships between (A) population size and mean fruit set per population, (B) population density and mean fruit set per population, (C) population size and mean population seed set, measured as the total seed weight/fruit in mg, and (D) population density and mean seed set. The open circle in A and C represents population BCE. Regression lines are drawn if the relationship is significant.

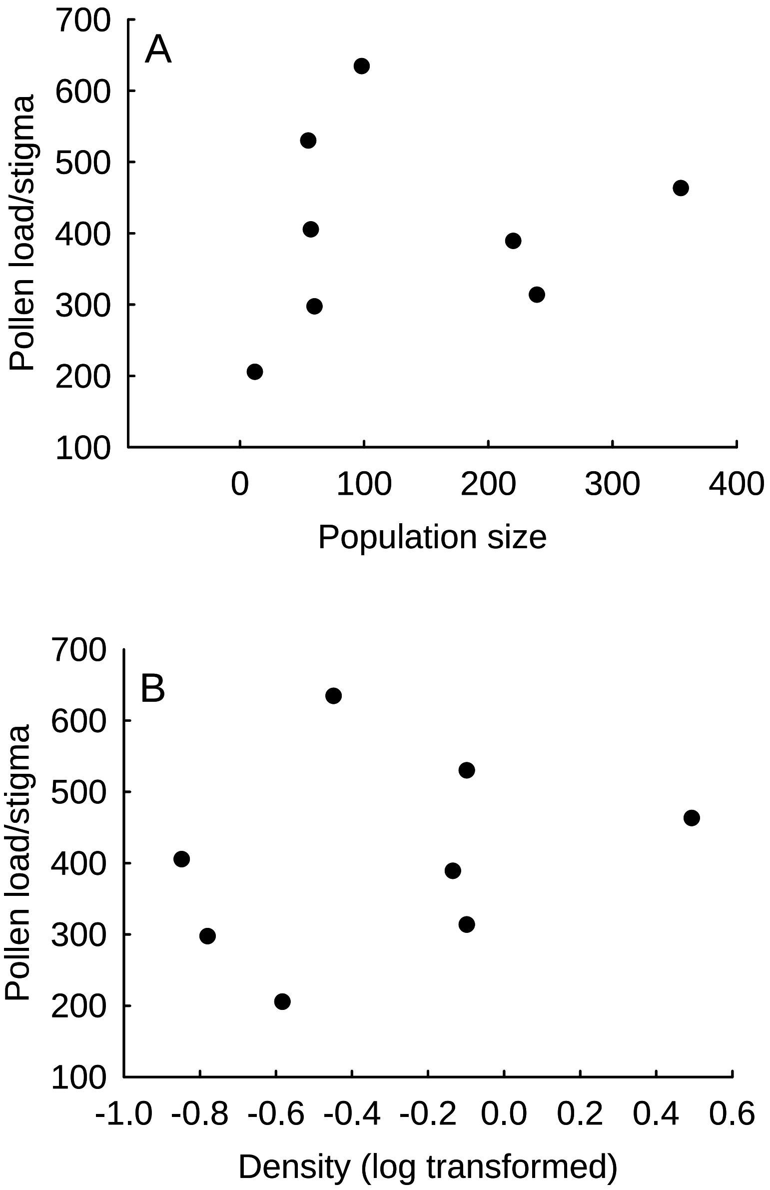

Figure 2.2. Relationships between mean stigmatic pollen load per populations and (A) population size and (B) population density.

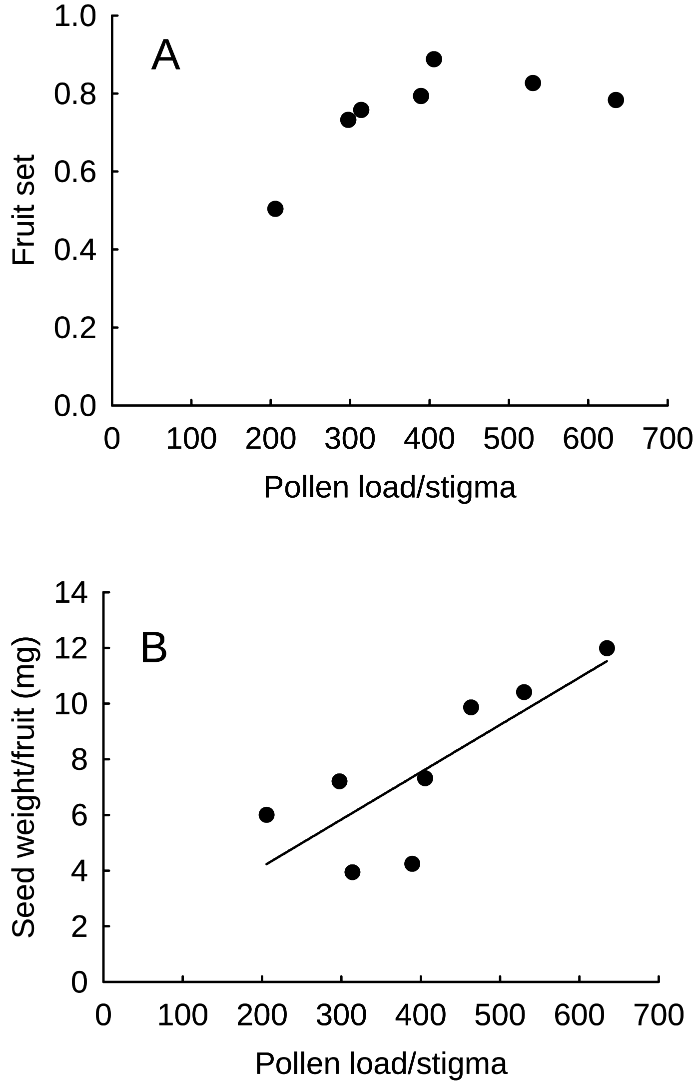

Figure 2.3. Relationships between mean pollen load/stigma sample and (A) mean fruit set and (B) mean seed set, measured as seed weight/fruit (mg), across eight study populations. Note only seven populations are included in A since WOM was not included in fruit set analyses. Regression lines are drawn if the relationship is significant.

CHAPTER 3

POPULATION SIZE AFFECTS THE MATING SYSTEM IN *SABATIA ANGULARIS* (GENTIANACEAE)[3]

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[3] Spigler, R.B., S.-M. Chang, and J.L. Hamrick. To be submitted to *American Journal of Botany*.

Abstract

Understanding how the mating system varies with population size in natural plant populations is critical for understanding their genetic and demographic fates. We examined how the mating system, characterized by the outcrossing rate, biparental inbreeding rate, effective number of pollen parents, and inbreeding coefficient varied with population size and density in natural populations of the biennial *Sabatia angularis* (Gentianaceae). In addition, we examined how variation in population size and density related to measures of genetic diversity, including allelic richness and heterozygosity. We found a significant, positive relationship between population size and outcrossing rate. Selfing rate was only about 7% in the largest population, but rose to 40% in the smallest population. Such high selfing rates could translate into lower population viability for small populations of *S. angularis*, a species known to experience significant inbreeding depression. A positive trend between density and biparental inbreeding rate suggests that high-density populations may have greater spatial genetic structure. Heterozygosity increased from the seedling to adult stage across all populations, consistent with selection against inbred individuals. This conclusion was further supported by significant excesses in adult heterozygosity in two populations with high selfing rates. Therefore, the consequences of increased selfing for population fitness will depend on the relative magnitude and consistency of inbreeding depression versus selection for outcrossed progeny in small populations.

Introduction

The mating system of plant populations can impact population dynamics by influencing the genetic composition of populations in subsequent generations (Hamrick *et al.* 1979; Loveless & Hamrick 1984; Hamrick & Godt 1996) and average individual fitness in populations

(Frankel & Soulé 1981; Oostermeijer *et al.* 2003). Because of this influence, population biologists are interested in understanding 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 rates, lower growth rates, and lower survival rates compared to offspring from larger populations (Menges 1991; Heschel & Paige 1994; Fischer & Matthies 1998a). Such fitness changes are redolent of the effects of inbreeding depression, the reduction in fitness of inbred progeny relative to outcross progeny, and often arise when the selfing rate increases in small populations.

Self-fertilization frequently increases in small populations of self-compatible, entomophilous species in response to decreased pollinator visitation (Antonovics & Levin 1980). Pollinator visitation is often reduced in such populations because pollinators are less attracted to small flowering patches (Levin & Anderson 1970; Sih & Baltus 1987; Kunin 1997). Some species compensate for reduced pollination through facultative selfing (Kalisz *et al.* 2004), but while this capability may buffer individuals against reproductive losses, it inevitably increases the selfing rate. Several studies have demonstrated increased selfing rates in small, natural plant populations (Routley *et al.* 1999; Robledo-Arnuncio *et al.* 2004; Hodgins & Barrett 2006).

Other aspects of the mating system, such as the rate of biparental inbreeding and the effective number of pollen parents (N_{ep}) (Ritland 1989) may also change with population size. In small populations, where individuals are typically more related to each other than expected by chance, biparental inbreeding can occur even with random mating (Wright 1931). Average N_{ep} per maternal plant, on the other hand, could decrease in small populations due to fewer potential fathers (Levin & Kerster 1969; Loveless & Hamrick 1984). These patterns have been observed in natural populations (Young & Brown 1998; Holderegger & Stehlik 1999; Robledo-Arnuncio *et al.* 2004) and may have subtle, but important, genetic and fitness consequences. Biparental inbreeding increases homozygosity and can contribute to inbreeding depression (Dole & Ritland 1993). In addition, fruits sired by a higher diversity of males may be selectively matured (Marshall & Ellstrand 1986), thereby increasing or maintaining genetic diversity. Increased genetic diversity generated by greater N_{ep} may subsequently translate into increased average population fitness by improving the chance of producing progeny best suited to the environment (Loman *et al.* 1988).

In addition to facing risks posed by changes in the mating system, small populations are often confronted by reduced genetic diversity (Frankel & Soulé 1981; Barrett & Kohn 1991; Young *et al.* 1996). Two genetic consequences occur as a result of small population size: a loss of heterozygosity due to increased inbreeding and a loss of allelic diversity through genetic drift (Ellstrand & Elam 1993). While the effects of genetic drift affect all populations, they are usually stronger in small populations (Wright 1931; Kimura & Crow 1964). In addition, small populations have a greater chance of accumulating fixed, deleterious alleles. Thus the effects of genetic drift on fitness and population viability are exacerbated in small populations (Lynch & Gabriel 1990; Lynch *et al.* 1995).

Conservation biologists are concerned with changes in the mating system in response to changes in population size because of the link between population size and viability (Soulé 1987; Lamont & Klinkhamer 1993). However, changes in the mating system could be associated with population density instead. Several studies have demonstrated increased self-fertilization rates for plants at low density (Murawski *et al.* 1990; Murawski & Hamrick 1991; Karron *et al.* 1995), possibly mitigated by pollinators that modify their movement patterns as density decreases (Heinrich 1979; Antonovics & Levin 1980). Biparental inbreeding is often related to density and their relationship may change depending on the level of spatial genetic structure within populations (Vekemans & Hardy 2004). Even the effective number of pollen parents may be associated with density, since density affects gene movement within populations (Levin & Kerster 1969; Antonovics & Levin 1980; Loveless & Hamrick 1984). Therefore, researchers interested in mating system changes with population size should also consider density.

In this paper, we examine how the mating system and genetic diversity vary with population size and density across natural populations of *Sabatia angularis* (Gentianaceae), a biennial native to the eastern U.S. and Canada. *S. angularis* was suitable for this study because natural populations vary widely in size and density (Spigler, unpublished data). We determined whether the outcrossing rate, biparental inbreeding rate, effective number of male parents, allelic richness, and heterozygosity correlate with either population size or density across eight *S. angularis* populations. Previous research demonstrated that this species experiences significant inbreeding depression (Dudash 1990; Spigler, unpublished data). Thus, variation in the mating system and genetic diversity in small populations could affect local population persistence.

Methods

Study Species and Sites

Sabatia angularis is an obligate biennial; seedlings germinate in early spring and overwinter as rosettes until the following spring when plants begin to bolt. In the study populations, plants produced showy, pink floral displays from July until late August. Inflorescences are terminal, comprised of flowers approximately 3-4 cm in diameter arranged in a panicle of cymes. 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, pers. obs.). Upon pollination, flowers develop into many seeded, dry dehiscent capsules and mature between September and November.

Although previous research suggests that this species is outcrossing (Dudash 1987), significant rates of inbreeding in natural populations might still occur. *S. angularis* is fully self-compatible (Dudash 1987, 1990), and despite being protandrous, autonomous self-pollination (autogamy) can occur when these phases overlap within a flower (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 upwards of 50 open flowers at a time, individuals may experience high levels of geitonogamy. Furthermore, the passive mode of seed dispersal in the species likely causes spatial family structure within populations, which, combined with local pollen dispersal, will lead to significant levels of biparental inbreeding (Wright 1943; Levin & Kerster 1969).

S. angularis is widely distributed in a variety of habitats including glades, marshes, rocky outcrops, old fields, roadsides, prairies, and serpentine barrens throughout its range in the eastern U.S. and Canada. We studied eight populations of *S. angularis* differing in size and density in North and South Carolina (Table 3.1). We assessed population sizes by counting all reproductive adults. Accurate counts of population size are feasible because natural *S. angularis* populations tend to be patchy and discrete. Mean density was estimated by dividing the number of adult individuals in each population by the area they covered.

In each of the eight study populations, we randomly selected 20 maternal individuals for the mating system analyses. The only exception was population COW2, which initially consisted of 15 flowering plants, but declined to eleven individuals due to mortality. We collected up to 15 naturally pollinated fruits prior to dehiscence from each chosen maternal plant per population. In February 2005, we planted seeds in flats at the University of Georgia Plant Biology Greenhouses and subjected them to cold stratification at 5°C for 60 days following an 12/12 hour day/night light regime. Our goal was to obtain tissue from twelve seedlings per maternal individual, so we planted extra seeds to account for the approximately 60% germination rate in the greenhouse (Spigler, unpublished data). For all planted seeds, we kept track of maternal 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 Allozyme Electrophoresis

We used eight to 15 seedlings per maternal individual per population for horizontal starch-gel electrophoresis. When possible, seedlings from different fruits were used to minimize the chance of sampling full sibs, which can affect the accuracy of mating system estimates (Schoen & Clegg 1984). We crushed approximately 400 mg of fresh tissue from each seedling in chilled mortars containing a polyvinylpyrrolidone-phosphate extraction buffer to extract enzymes (Mitton *et al.* 1979) and sand to grind the plant material. Extracts were absorbed onto Whatman 3 mm chromatography paper wicks and stored in microtiter plates at -70°C.

To estimate the mating system, we examined eight polymorphic loci using 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). Frequencies of the most common allele among loci ranged between 0.515 (± 0.068 SD) and 0.996 (± 0.003 SD) across the study populations (Appendix A). All stain recipes follow Soltis *et al*. (1983), except DIA (see Cheliak & 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

We estimated the following mating system parameters for each population using the multilocus mating system program MLTR (Ritland 1990): mutli-locus outcrossing rate (t_m), single-locus outcrossing rate (t_s), and correlation of outcrossed paternity (r_p). This program assumes two modes of mating: complete outcrossing and complete selfing. However, one can calculate biparental inbreeding rate (m_b) as the difference between the multi-locus and single-locus outcrossing rates for each population (Shaw *et al.* 1981; Ritland 1990). In addition, the effective number of pollen parents (N_{ep}) can be calculated as the inverse of r_p (Ritland 1989). We generated standard errors for the parameter estimates from MLTR using 1000 bootstrap iterations with family as the resampling unit. Progeny arrays were used to infer the maternal genotype in MLTR, from which F, the inbreeding coefficient of the parental generation, is estimated. For all parameter estimations, we constrained the pollen and ovule allele frequencies within a population to be equal. We ran MLTR separately for each population since allele frequencies were heterogeneous among populations (Ritland 2002). Only one population, COW2, had fewer individuals than the 200 recommended by MLTR. The consequence of this low sample size should be restricted to large standard errors for the mating system parameters.

We also estimated the following standard measures of genetic diversity within 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) per population. We used

POPGENE Version 1.32 (Yeh *et al.* 1997) to calculate these measures for the scored progeny genotypes and did the same for the parental generation using the maternal genotypes predicted by MLTR. We used the same polymorphic loci to calculate genetic diversity statistics as we used in the mating system analysis. We did not score additional, monomorphic loci. Therefore, the genetic diversity statistics presented are inflated relative to other studies that use a combination of monomorphic and polymorphic loci. Although this inflation makes comparisons between the genetic diversity of this species and other species difficult, it should not affect the comparisons made among populations in this study.

Relationships between population size and density and estimated mating system parameters were evaluated using Pearson Product Moment Correlation analysis. Specifically, we examined correlations between population size and density and t_m, m_b, N_{ep}, r_p, and F. Density and N_{ep} were log transformed to conform to model assumptions; all other parameters were normally distributed. We evaluated the significance of the mating system parameter estimates by calculating 95% confidence intervals as 1.96 multiplied by the standard deviation as given by MLTR. Similar correlations were performed for population size and density against A, A_e, H_o, and H_e for the progeny and maternal genotypes from each population.

Results

Population outcrossing rates were significantly correlated with population size (Fig. 3.1A). This effect was strong and positive. In contrast, the correlation between density and outcrossing rate was not significant (Fig. 3.1E). Influence statistics (DFFITS = -1.665) showed that population JRM had a disproportionately large effect on the relationship between density and outcrossing rate compared to the remaining populations. When this population was removed from the analysis, the correlation between outcrossing rate (t_m) and density among the remaining populations became significant ($r_7 = 0.79$, $p = 0.034$). A high correlation between population size and density (log transformed) across the eight study populations ($r_7 = 0.83$, $p = 0.011$), however, suggests that the relationships between population size, density, and t_m are not independent. Estimates for population outcrossing rates revealed that the study populations ranged from mixed-mating (0.601 ± 0.081) in population JRM to almost complete outcrossing (0.954 ± 0.028) in population WF. Mean outcrossing rate across all populations was 0.780 (± 0.119 SD).

Neither population size nor density was significantly correlated with biparental inbreeding (m_b) (Fig. 3.1B,F). A positive trend between density and biparental inbreeding, however, was apparent. Mean m_b was 0.075 (± 0.043 SD) and ranged from 0.012 (± .027 SD) in population BC2 to 0.131 (± 0.026 SD) in population WOM. Constructed confidence intervals reveled significant biparental inbreeding in six of the eight populations examined. Populations BC2 (95% CI for m_b: -0.041 to 0.065) and COW2 (95% CI for m_b: -0.050 to 0.110) did not exhibit significant biparental inbreeding.

The relationships between the effective number of pollen donors (N_{ep}) (log transformed) and population size and density are shown in Figure 1 (1c,1g). Neither population size nor density were significantly related to N_{ep}. Variation in its inverse, the correlation of paternity (r_p), is not shown but was also unrelated to population size ($r = 0.10$, $p = 0.818$) and density ($r = 0.20$, $p = 0.639$). Variation in N_{ep} among populations was fairly broad, ranging from approximately 9 in population WF to between one and two in population WOM. Mean N_{ep} was about 4 (3.654 ± 2.541 SD) and mean correlation of paternity, r_p, was 0.374 (± 0.188 SD).

We did not find a significant correlation between F, the inbreeding coefficient for the parental generation, and either population size or density (Fig. 3.1D,H). The mean inbreeding coefficient across all study populations was -0.070 (± 0.125) and ranged from -0.200 in both populations BC2 (± 0.029 SD) and COW2 (± 0.089 SD) to 0.128 (± 0.136 SD) in population BC1. In all but two populations, F was not significantly different from zero, indicating that inbreeding was not significant in reproductive individuals in the majority of populations. Instead, F values were significantly lower than zero for populations BC2 and COW2, suggesting that these populations have an excess of heterozygotes in the parental generation relative to equilibrium predictions. Across all populations, there was a trend showing increased heterozygosity in the parental generation compared to the progeny generation (Table 3.2). None of the genetic diversity measures examined, however, had a significant relationship with either population size or density (Table 3.3).

Discussion

Outcrossing rate was significantly and positively correlated with population size across natural populations of *Sabatia angularis*. This relationship was strong; almost half of the variation in outcrossing rate could be explained by variation in population size alone. Similar results were found for Scots pine (Robledo-Arnuncio *et al.* 2004) and the herbs *Narcissus triandrus* (Hodgins & Barrett 2006) and *Aquiliegia canadensis* (Routley *et al.* 1999). Increased selfing in small populations of *S. angularis* may reflect changes in the proportion of outcross pollen received relative to self-pollen deposited via autonomous self-fertilization. Well documented evidence suggests that pollinator visitation rates decline in small populations (e.g., Levin & Anderson 1970; Sih & Baltus 1987; Kunin 1997). For many self-compatible species, autonomous self-fertilization provides reproductive assurance when pollinators are scarce (Kalisz *et al.* 2004). If the ratio of self-pollen to outcross pollen on stigmas varies in proportion to pollinator visitation rates across population sizes, we would expect to see a positive correlation between population size and outcrossing rate. Routley *et al.* (1999) concluded that this mechanism accounted for higher selfing rates in small populations of the self-compatible, autogamous plant *Aquiliegia canadensis*. *S. angularis* is similarly capable of autonomous self-pollination (Spigler, unpublished data). Experimental floral manipulations involving pollinator exclusion and emasculation treatments could be used to discriminate between the quantities of outcross and autogamous pollen loads on *S. angularis* individuals in small and large populations (Schoen & Lloyd 1992) and thus to evaluate whether this mechanism is responsible for the significant correlation between population size and outcrossing rate in this species.

We can not rule out the possibility that variation in outcrossing rate among the study populations is caused by variation in population density rather than population size. Density has been shown to affect the mating system in several tropical tree species (Murawski *et al.* 1990; Murawski & Hamrick 1991), but its effects appear to be less consistent among herbaceous plants (van Treuren *et al.* 1993a; van Treuren *et al.* 1994; Lu 2000). Few studies have examined the effects of population size and density simultaneously, making it difficult to decipher whether population size or density serves as a better predictor for outcrossing rate (but see van Treuren *et al.* 1993a). Unfortunately, the high correlation between population size and density in this study retards our ability to make this distinction. We suspect that density affects outcrossing rate merely through its strong correlation with population size and that population size influences outcrossing rate more directly. This assertion is supported by the significant relationship between population size and outcrossing rate in contrast to no relationship between density and outcrossing rate. Density was significant when population JRM was removed as a statistical outlier, but we are not aware of a biological justification for its removal and therefore, suggest that population size is more appropriate. Evidence also comes from comparing the outcrossing rate of population JRM to that for several other populations. Population JRM is similar in size to populations LI1 and BC2 but similar in density to population WF, which is four times greater in size than JRM. The outcrossing rate of JRM is similar to the outcrossing rates of LI1 and BC2 while it is significantly different from WF (Spigler, unpublished data). Experimental manipulations isolating the effects of population size and density would best reveal which factor affects the outcrossing rate in this species, but this case is consistent with our conclusion that population size is a better predictor of outcrossing rate for this species.

Prior to this study, little was known about the mating system of *Sabatia angularis*. The only estimate of outcrossing rate came from a study of one population of *S. angularis* in which Dudash (1987) estimated the outcrossing rate to be 93% using a method comparing the fitness of selfed, outcrossed, and naturally pollinated progeny (Charlesworth & Charlesworth 1987). This method is indirect, and a potential problem is that the estimate is affected by the quantity and quality of pollen used. However, Dudash's estimate is well within the range of our genetically-based estimates. Interestingly, the estimate of a 93% outcrossing rate came from a population where *S. angularis* was described as "abundant" (Dudash 1987) and thus is consistent with our conclusion that large populations of *S. angularis* have higher outcrossing rates. Our data confirm Dudash's conclusion that *S. angularis* is a primarily outcrossing species (Schemske & Lande 1985), but we estimate an average outcrossing rate of 78%, about 15% lower than that reported by Dudash. The true average outcrossing rate of this species may be even lower than 78%, because selfed offspring from the study populations have significantly lower germination rates than outcrossed offspring (Spigler, unpublished data). Consequently, seedlings included in the genetic analysis may have been overrepresented by outcrossed offspring.

None of the remaining mating system parameters examined varied significantly with population size. However, we noticed that biparental inbreeding tended to increase with density. Significant biparental inbreeding was found in almost all study populations and likely reflects a combination of localized seed and pollen dispersal. Seeds of *S. angularis* disperse passively from fruits that remain attached to maternal plants, and presumably, most seeds remain near the maternal plant (Dudash 1991). Furthermore, the majority of pollinator flights between plants of *S. angularis* occur within 1 m of a given plant (Dudash 1987). A positive trend between density and biparental inbreeding might arise if genetic structure increases in high density populations of *S. angularis*, a result that has been seen in other studies (e.g. Coates *et al.* 2006; Hodgins & Barrett 2006). Gene movement, however, is likely greater than 1 m due to pollen carryover (Schaal 1980). Consequently, high pollen carryover, overlapping seed shadows among plants at high densities (Vekemans & Hardy 2004; Gapare & Aitken 2005), or both may have limited the relationship between density and biparental inbreeding seen in this study. Alternatively, the weak relationship may be an artifact of the small number of populations included and, accordingly, reduced statistical power. Evidence presented here suggests the latter scenario. Consistent with a positive relationship, the population with the highest density, WOM, exhibited the highest biparental inbreeding rate while the two smallest populations exhibited the lowest.

Estimates of the effective number of male parents (N_{ep}) suggest that when *S. angularis* plants outcross, they receive pollen from approximately four pollen donors, on average, assuming each donor contributes equally to the pollen pool. The actual number of pollen donors per individual, however, is likely much higher. First, the underlying assumption of equal siring probability is often violated (e.g. Smyth & Hamrick 1984). Second, programs such as MTLR significantly underestimate the number of male sires per plant compared to direct paternity analyses (Smouse & Sork 2004; Pardini & Hamrick 2007). Regardless, the present N_{ep} estimates allow meaningful comparisons among populations. While neither population size nor density were significantly associated with N_{ep}, one population, WF, had a considerably greater number than the remaining populations and, noticeably, had the highest outcrossing rate as well. This population was located near an apple orchard that was home to a modest honey bee apiary. It is quite possible then, that high bee activity in this population greatly promoted gene movement and generally served to increase the outcrossing rate.

Despite the significant positive relationship between population size and outcrossing rate estimated from the progeny, no relationship was evident between population size and the parental inbreeding coefficient, F. Furthermore, the majority of populations did not have inbreeding coefficients significantly different from zero even though most populations had significant levels of selfing. Several explanations could account for these apparent discrepancies. First, it is possible that selfing has not been consistent over multiple generations or strong enough to reduce heterozygosity within populations. Repeating this study over subsequent years would reveal whether inbreeding these populations varies over time. Alternatively, natural selection between the seedling stage, from which we obtained our population level outcrossing rates, and the adult stage may have favored heterozygous genotypes, thus preserving heterozygosity and disguising any relationship between population size and the inbreeding coefficient (Clegg & Allard 1973; Schaal & Levin 1976; Farris & Mitton 1984). Several lines of evidence suggest that this may occur in *S. angularis* populations. First, selfed progeny of *S. angularis* have significantly lower survival than outcrossed progeny (Dudash

1990; Spigler, unpublished data). Second, both population BC2 and COW2 show a significant excess of heterozygotes in the parental generation, despite having the highest selfing rates. Lastly, our results reveal that heterozygosity is higher for maternal individuals than their progeny across all populations examined (Table 3.2).

The apparent independence of genetic diversity and population size demonstrated in this study contrasts with the current paradigm that small populations ought to have lower heterozygosity due to inbreeding and reduced allelic diversity via genetic drift (Frankel & Soulé 1981; Barrett & Kohn 1991; Ellstrand & 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). Yet no relationship may be expected if small populations represent fragments of previously large, continuous populations, such as was found for the perennial *Antirrhinum valentinum* (Mateu-Andrés & Segarra-Moragues 2000). This scenario unlikely applies to *S. angularis*, given its biennial life history. Alternatively, gene flow (Richards 2000) or a persistent seed bank (Levin 1990; McCue & Holtsford 1998) would buffer the loss of genotypic and allelic diversity in small populations in *S. angularis*. Evidence for a seed bank in *S. angularis,* however, is equivocal (Dudash 1987; C. Baskin, pers. comm.), and without knowledge of gene flow in this species an explanation for the association between population size and genetic diversity seen in this study remains unknown.

The main goal of this study was to assess the relationship between population size and the mating system in natural populations of the biennial *Sabatia angularis.* Smaller populations of *S. angularis* have lower outcrossing rates, with selfing rates as high as 40% in the smallest population examined. This figure is more than six times greater than the selfing rate of the largest population included in this study, and may even represent an underestimate of the actual selfing rate in natural populations given that early selection occurs against selfed progeny. Because significant inbreeding depression also occurs for survival and reproduction in *S. angularis* (Dudash 1990; Spigler, unpublished data), increased selfing in small populations might have critical implications for the viability of small populations of this species. These implications might include lower population growth rates and decreased extinction times, such as was found for populations of *Gentiana pneumonanthe* that experienced inbreeding (Oostermeijer 2000). The excess of heterozygotes seen in two *S. angularis* populations with high selfing rates, however, suggests that the negative effects of inbreeding depression in this species may, in part, be mitigated by selection for heterozygote genotypes as plants mature. Monitoring the mating system and genetic diversity over several generations would further our understanding of the consequences of changes in the mating system with population size in *Sabatia angularis*.

Table 3.1. List of study populations including location, population size, and density (individuals/m^2).

Population	**Location**	**Population Size**	**Density**
COW2	Macon Co, NC	15	0.136
JRM	Clay Co., NC	55	0.797
LI1	Pickens Co., SC	57	0.142
BC2	Clay Co., NC	60	0.166
LI2	Pickens Co., SC	76	0.247
BC1	Clay Co., NC	207	0.364
WF	Macon Co, NC	220	0.733
WOM	Clay Co., NC	355	3.114

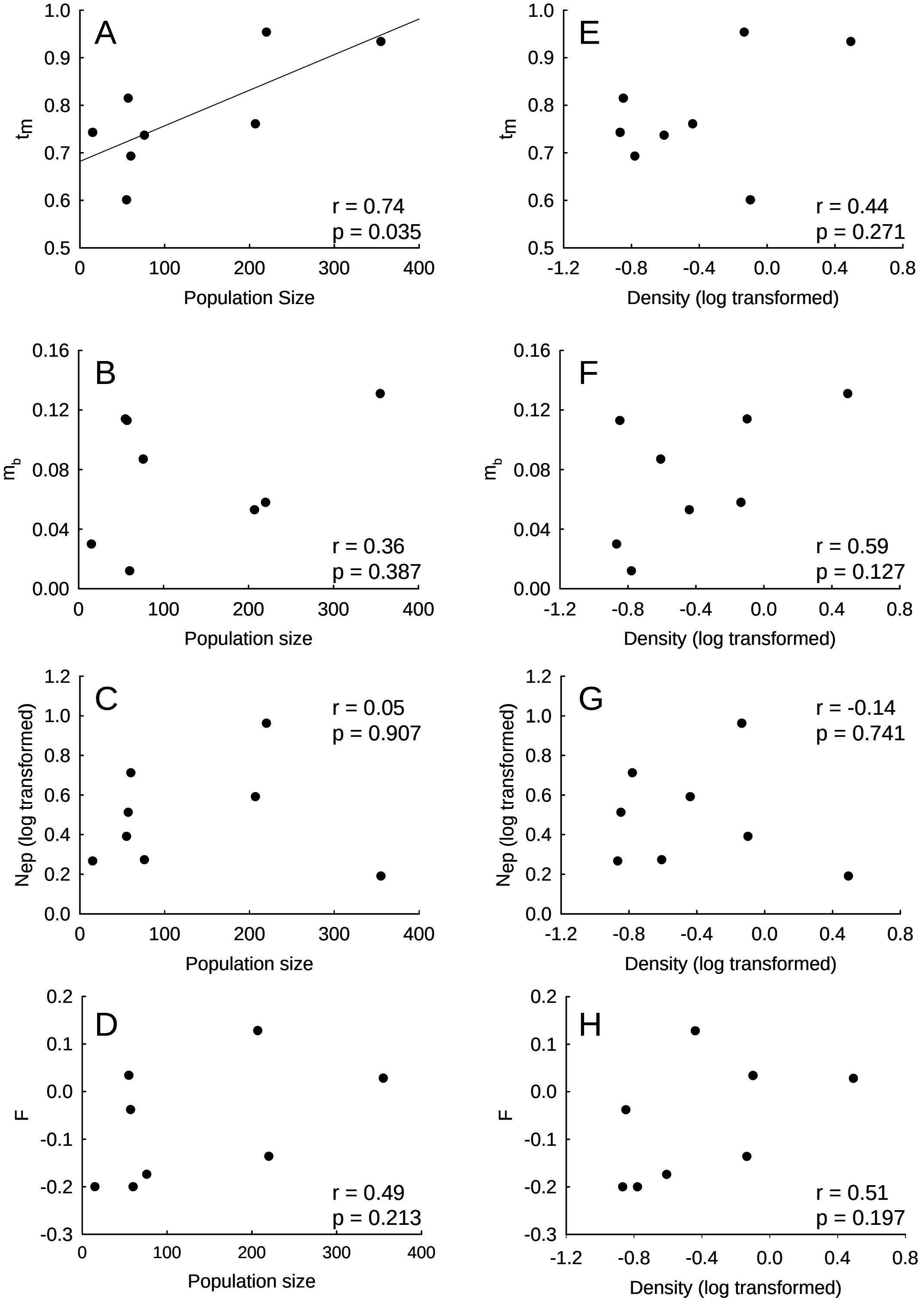
Table 3.2. Genetic diversity statistics for each population for (I) maternal individuals and (II) progeny. A = mean number of alleles/locus; A_e = mean effective number of alleles/locus; H_o = mean observed heterozygosity; H_e = expected heterozygosity. One standard deviation is shown in parentheses.

Population	**A**	**A_e**	**H_o**	**H_e**
I. Maternal Plants				
COW2	2.125 (0.641)	1.492 (0.499)	0.375 (0.324)	0.285 (0.213)
JRM	2.500 (0.707)	1.476 (0.419)	0.257 (0.208)	0.280 (0.210)
LI1	2.500 (0.756)	1.560 (0.610)	0.319 (0.313)	0.289 (0.242)
BC2	1.750 (0.707)	1.293 (0.302)	0.224 (0.219)	0.196 (0.184)
LI2	2.500 (0.756)	1.670 (0.392)	0.463 (0.266)	0.375 (0.177)
BC1	1.875 (0.991)	1.320 (0.429)	0.163 (0.190)	0.186 (0.225)
WF	2.250 (0.707)	1.474 (0.521)	0.306 (0.304)	0.258 (0.236)
WOM	2.375 (0.518)	1.620 (0.498)	0.369 (0.262)	0.337 (0.211)
Mean	2.234 (0.295)	1.488 (0.132)	0.309 (0.095)	0.276 (0.064)
II. Progeny				
COW2	2.500 (0.535)	1.439 (0.435)	0.211 (0.208)	0.256 (0.193)
JRM	2.500 (0.756)	1.429 (0.415)	0.163 (0.159)	0.248 (0.210)
LI1	2.500 (0.456)	1.462 (0.440)	0.187 (0.182)	0.261 (0.217)
BC2	2.000 (0.756)	1.201 (0.224)	0.099 (0.106)	0.144 (0.147)
LI2	2.625 (0.916)	1.737 (0.449)	0.314 (0.213)	0.385 (0.185)
BC1	2.000 (0.926)	1.295 (0.448)	0.118 (0.162)	0.162 (0.220)
WF	2.375 (0.519)	1.388 (0.485)	0.209 (0.263)	0.213 (0.223)
WOM	2.500 (0.535)	1.552 (0.505)	0.259 (0.290)	0.291 (0.230)
Mean	2.375 (0.241)	1.438 (0.161)	0.195 (0.071)	0.245 (0.076)

Table 3.3. Pearson Product Moment Correlations for the relationships between population size and density (log transformed) and the 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) calculated for (I) progeny and (II) maternal individuals. None of the correlation coefficients presented are significant at the p = 0.05 level. (n = 8 populations)

	A	A_e	H_o	H_e
I. Progeny				
Population size	-0.105	0.071	0.193	0.057
Density	0.176	0.203	0.262	-0.029
II. Maternal plants				
Population size	0.007	0.133	-0.061	0.071
Density	0.292	0.272	0.026	0.266

Figure 3.1. Relationships between population size and (A) multilocus outcrossing rate (t_m), (B) biparental inbreeding rate (m_b), measured as the difference between the multilocus and single locus outcrossing rates, (C) effective number of male parents (N_{ep}) (log transformed), and (D) parental inbreeding coefficient, F, and between density and (E) t_m, (F) m_b, (G) N_{ep}, and (H) F. Regression lines are drawn where significant.



A
r = 0.74
p = 0.035
B
r = 0.36
p = 0.387
C
r = 0.05
p = 0.907
D
r = 0.49
p = 0.213
E
r = 0.44
p = 0.271
F
r = 0.59
p = 0.127
G
r = -0.14
p = 0.741
H
r = 0.51
p = 0.197
t_m
m_b
Nep (log transformed)
F
Population Size
Population size
Density (log transformed)

Figure 3.1.

CHAPTER 4

THE MAGNITUDE AND PERVASIVENESS OF INBREEDING DEPRESSION ACROSS SIX NATURAL POPULATIONS OF THE NATIVE BIENNIAL *SABATIA ANGULARIS* (GENTIANACEAE)[4]

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[4] Spigler, R.B. and S.-M. Chang. To be submitted to *Evolution.*

Abstract

Inbreeding depression and its consequences have classically been couched in evolutionary terms. However, the same reductions in reproduction, growth, and survival caused by inbreeding depression also have the potential to impact demographic dynamics. To improve our understanding of inbreeding's capacity to affect demographic processes, investigations concerning the prevalence of inbreeding depression across multiple populations within species are needed. This study examines the prevalence and magnitude of inbreeding depression across six populations of the self-compatible biennial *Sabatia angularis* (Gentianaceae). We performed experimental self and outcross pollinations on maternal individuals *in situ* and examined performance of offspring in two environments: a common garden and the natural, maternal environment. Two types of outcross pollinations were included: pollinations between individuals in the same population and pollinations between populations. We examined whether *S. angularis* experiences inbreeding depression across a variety of traits and life stages and whether these results were consistent across all populations. We also evaluated the presence of outbreeding depression. We found significant inbreeding depression for germination, date of seedling emergence, rosette size, and survival across all populations in both environments. These effects culminated in significant inbreeding depression for a composite measure of fitness. Inbreeding depression for fitness averaged 43% in the common garden environment and 33% across maternal environments. We did not find evidence for outbreeding depression, but instead found evidence to suggest that outcross pollinations between populations might facilitate the colonization of new sites. Inbreeding depression was remarkably consistent across populations in their maternal environment, supporting the notion that inbreeding depression is pervasive in natural *S. angularis* populations. Furthermore, the magnitude of inbreeding depression and the

stages at which it manifests suggests that inbreeding has the potential to affect the demography of *S. angularis*.

Introduction

Inbreeding is a common phenomenon in natural plant populations, with approximately 40% of flowering plant species capable of self-fertilization (Richards 1997), the extreme form of inbreeding. An even greater proportion of plant species may inbreed inadvertently by mating with related individuals as a consequence of limited pollen and seed dispersal in natural populations (Levin & Kerster 1969; Antonovics & Levin 1980). The prevalence of inbreeding among plants inevitably leads to opportunities for the expression of inbreeding depression, the reduction in fitness of inbred progeny relative to outbred progeny, in natural populations. Inbreeding depression (hereafter, "ID") has been found in a majority of plant species in which it has been explicitly examined (Charlesworth & Charlesworth 1987; Crnokrak & Roff 1999; Keller & Waller 2002), and at least one measure based on 15 plant species estimates mean ID for plants around 33% (Crnokrak & Roff 1999). It is no wonder, therefore, that ID is considered the primary selective force in the evolution of plant mating systems (Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Uyenoyama *et al.* 1993; Barrett & Harder 1996).

The same reductions in reproduction, growth, and survival from ID that affect evolutionary processes also have the potential to affect ecological and demographic ones, including plant-pollinator and plant-herbivore interactions (Carr & Eubanks 2002; Ivey *et al.* 2004), population growth rates, and even rates of local extinction (Mills & Smouse 1994; Frankham 1995; Oostermeijer 2000; Brook *et al.* 2002). These effects may be particularly important for small populations, which can have increased rates of selfing (Raijmann *et al.* 1994; Routley *et al.* 1999; Robledo-Arnuncio *et al.* 2004; Hodgins & Barrett 2006).

Yet while studies on the consequences of ID for mating system evolution abound, studies focusing on the ecological and demographic consequences are comparatively rare. This underappreciation may be related to the once-held notion that ID ought to have little impact on population dynamics. There are two main foundations for this perception. First, Lande (1988) suggested that genetic processes should have relatively negligible effects on population dynamics compared to the effects of demographic stochasticty (see also Pimm *et al.* 1989). This argument was based upon the theory that natural selection should effectively purge ID from populations (Lande & Schemske 1985; Barrett & Charlesworth 1991; Husband & Schemske 1996) and upon a lack of adequate support at the time for a link between genetic diversity and population fitness. Current evidence for purging, however, is equivocal (Byers & Waller 1999; Miller & Hedrick 2001; Crnokrak & Barrett 2002), and although there is some empirical evidence for the purging of lethal recessive alleles, mildly deleterious alleles might persist (Charlesworth & Charlesworth 1987; Hedrick 1994; Wang *et al.* 1999; Keller & Waller 2002). Furthermore, purging of lethal alleles can take several generations to occur, during which important ecological interactions and demographic dynamics are taking place. In addition, there is now strong evidence that population fitness is correlated with heterozygosity (Oostermeijer *et al.* 1995; Fischer & Matthies 1998b; Lienert *et al.* 2002; Reed & Frankham 2003; Leimu *et al.* 2006).

Second, underappreciation of the demographic consequences of inbreeding may also be related to evidence of significant variation among populations in the presence and magnitude of inbreeding depression. This evidence has cast doubt on our ability to make generalizations about the consequences of ID for population dynamics. Indeed, ID has been shown to vary with environmental conditions (e.g. Dudash 1990; Ramsey & Vaughton 1998; Hayes *et al.* 2005), mating history (e.g. Holtsford & Ellstrand 1990; Fishman 2001; Mustajärvi *et al.* 2005; Goodwillie & Knight 2006), and demographic factors such as population size (Bataillon & Kirkpatrick 2000; Paland & Schmid 2003). Yet, while these factors are important to consider when comparing levels of ID across multiple populations, variation among populations in inbreeding depression is not ubiquitous (van Treuren *et al.* 1993b; Groom & Preuninger 2000), nor does such variation suggest that inbreeding depression is not a stable enough force to influence population dynamics.

Past studies attempting to quantify the population-level consequences of inbreeding have focused on the effects of reduced genetic diversity or determined the number of lethal equivalents or genetic load (e.g., Sorensen 1969; Franklin 1972; Polans & Allard 1989). More recently, researchers have begun to explore the demographic effects of inbreeding explicitly by incorporating inbreeding depression into demographic models and simulations (Mills & Smouse 1994; Frankham 1995; Oostermeijer 2000; Brook *et al.* 2002). In order for us to understand the applicability of these models to plant populations and species in general, surveys of inbreeding depression across multiple populations, such as this one, remain a primary objective.

The main goal of this study was to determine the prevalence and magnitude of inbreeding in the biennial *Sabatia angularis* (L.) Pursh (Gentianaceae) to determine whether inbreeding depression is a strong and consistent force across natural populations. *S. angularis* is appropriate for this study as it exhibits a mixed mating system (see Chapter 3) and has been shown to exhibit inbreeding depression in at least one natural population from crosses performed in the greenhouse (Dudash 1990). Specifically, we asked the following questions: (1) Do outcross progeny outperform self progeny? (2) Which traits and life stages exhibit inbreeding depression? (3) Are these results consistent across six natural populations? This investigation represents a first step toward establishing the potential for demographic consequences of inbreeding depression in this species.

Methods

Study Species & Sites

Sabatia angularis is an herbaceous biennial found in a variety of habitats including glades, marshes, old fields, roadsides, and prairies throughout the eastern United States and southeastern Canada (USGS 2003). *S. angularis* begins its biennial life-cycle in spring when seeds germinate. Plants develop into rosettes and overwinter until the following summer when they begin to bolt. From July until late August, plants produce displays of showy, pink flowers, which develop into many seeded, dry dehiscent capsules from September to November. A generalist suite of pollinators, including leaf-cutter bees (Megachilidae), sweat bees (Halictidae), andrenid bees (Andrenidae), small carpenter bees (Anthophoridae), and hover flies (Syrphidae) visit the flowers for their pollen reward (Dudash 1987; Spigler, pers. obs.).

Individuals of this species are self-compatible (Dudash 1987, 1990) and self-pollination can occur through geitonogamy and autogamy. Despite being protandrous, individuals produce flowers continuously during their flowering period. A medium-sized individual can have as many as 50 flowers open at a time, providing plenty of opportunities for geitonogamous pollination. Furthermore, male and female phase within a flower are rarely discrete, and autogamy can occur when male and female phase overlap (Spigler, unpublished data). Previous work revealed that the average rate of selfing across natural populations is 22%, but selfing rates in individual populations can range from 5% to potentially greater than 40% (Spigler, unpublished data). In addition, most populations experience significant rates of biparental inbreeding (Spigler, unpublished data). The average biparental inbreeding rate found previously in natural *S. angularis* populations is 7.5% but can be twice as high in some populations (Spigler, unpublished data). Thus, opportunities for the expression of inbreeding depression in natural populations of this species are frequent.

Experimental Cross Pollinations

Inbreeding depression is typically assessed by comparing the relative fitness of selfed versus outcross progeny. To accomplish this, experimental crosses were performed *in situ* during the 2005 flowering season in six natural populations located in North and South Carolina (BC1, BC2, JRM, LI1, LI2, WF). In each population, we randomly selected up to 25 individuals and covered them with pollinator exclusion bags made from bridal veil prior to flowering. Once plants began to flower, we applied the following pollination treatments: self pollen (S), “near” outcross pollen from another individual within the population (ON), or “far” outcross pollen from an individual located in another population (OF). Every flower on a given maternal plant received one of the three pollination, or “cross”, treatments. We included the second outcross treatment, OF, for two reasons. First, it allows us to detect local adaptation within populations by examining whether OF progeny have significantly lower fitness than ON progeny, a phenomenon referred to as “outbreeding depression” (Price & Waser 1979; Lynch 1991; Waser & Price 1994). Second, the OF treatment allows for a more thorough interpretation of comparisons between S and ON progeny. If S progeny are less fit than ON progeny, we can conclude that there is inbreeding depression. However, if the two treatments are equal, we cannot determine unambiguously whether this is due to a lack of inbreeding depression or due to depressed genetic diversity within populations, which can render outcross pollinations within populations genetically equivalent to inbreeding. If equivalent fitness between S and ON were due to the latter scenario, we would expect bringing in novel genes from another population, i.e. the OF treatment, to increase fitness (Levin 1984; Fenster 1991).

We performed experimental pollinations on maternal individuals every other day in each population. This routine was a logistic necessity caused by the physical distance between the NC and SC populations. However, because flowers last for at least four days and enter male phase first, we were able to emasculate flowers upon each visit prior to stigma receptivity. For the ON and OF treatments, we assigned pollen donors haphazardly to study plants according to which plants had pollen available that day. Individual flowers received pollen from only one donor, but outcross pollinations across flowers on a given maternal individual represented a diversity of pollen donors. To account for possible donor effects on fruit and seed set, we kept track of donor identity for each pollination. We pollinated flowers by rubbing dehisced anthers directly onto the stigmatic surface of each flower until the stigma was visibly covered with the yellow pollen. Pollination treatments on each maternal plant were carried out in sets of three (S, ON, OF); within each set, the cross treatment was randomly ordered. We continued to apply the experimental crosses according to repeated randomized sets until all flowers on a maternal individual had been pollinated. The number of pollinations performed on a given maternal individual ranged from three to 54. Since plants can allocate more resources to earlier fruits, regardless of pollen source (Medrano *et al.* 2000), this design avoided confounding pollination date with cross treatment.

Unfortunately, a deadly fungal pathogen in the natural populations ultimately reduced our sample size of 25 individuals per population to 15. Sample sizes were smaller than 15 in three populations. Only seven families could be represented in population BC2 because plants were unexpectedly mowed toward the end of the flowering season. Mortality in populations JRM and WF decreased sample sizes to 10 and eight families, respectively.

Fruit and Seed Set

Each fruit on given maternal plant corresponds to a pollination, or cross, treatment. Fruit set was scored as a binomial variable; either a particular cross resulted in a mature fruit or it did not. We then collected mature fruits to estimate seed set for each individual fruit. Seed set is typically assessed as the number of seeds per fruit. However, counting seeds per fruit in this species is impractical since the seeds are extremely small (approximately 0.025 ± 0.008 SD mg/seed) (Spigler, unpublished data). Instead, we used fruit (capsule) mass as a proxy for seed set. Fruit mass is significantly and highly correlated with total seed mass per fruit ($r_{71} = 0.985$, $p < 0.0001$), and seed mass is significantly correlated with seed number ($r_{11} = 0.88$, $p = 0.003$) (Spigler, unpublished data). We estimated seed set, measured as fruit mass, for up to 10 fruits per cross treatment per individual.

Early Life History Traits

In February 2006, we planted seeds into flats containing an enriched pine bark/vermiculite (60:40) soil mixture. We then subjected flats to cold stratification for 12 weeks at 5°C under a light regime mimicking natural daylight cycles over that time period, beginning at 10 hours of daylight and ending at 14 hours by the 12th week. Our goal was to obtain 10 replicate seedlings per cross type for each maternal individual (hereafter, “family”) in populations BC1, BC2, LI1, and LI2, represented in both field experiments (see below), and 5 replicate seedlings per cross per family for populations WF and JRM. To account for an approximately 60% germination rate in the greenhouse (Spigler, unpublished data), we planted five seeds per cell per flat in order to obtain one seedling. Each cell, therefore, represents a replicate, and contained seeds from only one fruit. Up to five fruits per cross type per family were represented among the replicates where possible, and we kept track of fruit identity. Placement of replicates in flats was completely randomized. We examined flats weekly for germination and recorded emergence date to determine if there were differences in the timing of germination among cross types. Germination was scored as the number of seeds germinated per cell, or replicate, out of the five seeds planted in each cell. Seedlings were kept in the greenhouse until fall, when they had fully formed into rosettes. We measured the diameter of each rosette to examine differences in plant size among cross types. To estimate initial growth rates of rosettes, we divided rosette size by the number of days since emergence. Emergence date, rosette size, and growth rate represent averages per cell when more than one seed per cell germinated. Prior to transplanting, we randomly thinned seedlings to one per cell. We chose to wait until then to reduce the number of rosettes to one in order to maximize the chances of maintaining our original sample size goal in the following field experiments.

Common Garden

In October 2006, we transplanted rosettes into a common garden plot at the University of Georgia Plant Sciences Farm near Watkinsville, GA. Five replicate rosettes per cross per family for each population were randomly arranged into five blocks such that each cross type per family was represented once in each block. For some families, replication per cross was lower due to mortality in the greenhouse and/or low germination. Plants were planted in 0.5m x 0.5m grids within each block, which represents the highest densities seen in natural populations (Spigler & Chang, unpublished data). We watered plants immediately after planting and weeded the plots after the first month. After this, however, no additional water or weeding was performed. Four months after planting, we scored individuals for survival.

Maternal Sites

In November 2006, we transplanted a second set of rosettes from four of the six populations back into their respective maternal environment ("maternal site"). We excluded two populations (JRM and WF) from the maternal site plantings since their locations are often disrupted by mowing and hiking. Fifteen families per population were represented in three of the four populations; in population BC2, only replicates of seven families could be represented. At each site, we planted up to five replicates per cross type per family into three blocks in populations BC1, LI1, and LI2 and into a single block in population BC2 because of the small sample size for that site. We spaced plants in 0.5m x 0.5m grids within each block in populations BC2, LI1, and LI2 and in 0.25m x 0.25m grids in population BC1 due to limited space in that population. At four months post-transplanting, we assessed survival at each maternal site.

Fitness

S. angularis is a biennial; the time between seed set of one generation to seed set of the next takes two years. The data presented here, from seed set to survival, represent approximately 17 months of this life cycle. We calculated a composite measure of fitness for up to 17 months in each environment for each cross type using a multiplicative fitness function (*sensu* Dudash 1990). We calculated this measure for each fruit that matured. In many cases, we planted multiple seeds per fruit. Therefore, we calculated fitness by multiplying the number of seeds for that fruit, mean germination rate of seeds from that fruit, and mean survival of those individuals. Using this function, "fitness", as it is referred to within this paper, represents the number of individuals expected to survive to 17 months as a result of self- or outcross-pollination of a given flower. Fitness was calculated for each environment separately.

Statistical Analyses

We evaluated the effect of cross type (OF, ON, S) on fitness and the following traits and life history stages as they were measured above: fruit set, seed set, germination, emergence date, rosette diameter, initial growth rate, and survival. We used a mixed model approach to evaluate the effect of cross type on each of the measured traits and fitness. Cross type was included as a fixed effect. Population, family nested within population, and block or flat, where appropriate, were included as random effects. We chose to treat population as a random effect because we believe that the populations included in this study represent typical *S. angularis* populations. We carried out a mixed-model ANOVA using Proc MIXED in SAS version 9.1 (1999) to evaluate the effect of the fixed and random effects on seed set (measured as fruit weight, log transformed), emergence date, rosette diameter (log transformed), and initial growth rate (log transformed). To examine the effect of cross type and all random effects on fruit set, germination, survival, and fitness, we used generalized linear mixed models in Proc GLIMMIX. We considered fruit set, germination and survival to follow a binomial distribution. Since fitness represents a count of offspring that have survived per cross, we treated this variable as following a Poisson distribution. Issues of overdispersion associated with these distributions are automatically accounted for in the GLIMMIX procedure (Littell *et al.* 1996). Analyses for survival and fitness were carried out separately for the common garden and maternal sites. Because pollen donor did not account for significant variation in either fruit or seed set (unpublished data), we did not include this variable in the analyses. For analyses in which cross type was significant, we proceeded by performing Tukey's HSD post hoc tests to determine which treatments were significantly different from one another.

In addition to evaluating whether the main effect of cross type significantly affected the measured fitness traits, we were interested in whether these effects were consistent across populations. This can be achieved by evaluating the significance of the interaction between population and cross treatment. Following Johnston and Schoen (1994), we used the log of each response variable when testing for the significance of the interaction term. Using log transformed data allows one to test for a difference in the magnitude of inbreeding depression (ID), measured as the difference between cross treatment estimates. Because the interaction term in these analyses is a random variable, we evaluated its significance with a likelihood ratio test with 1 d.f. (Littell *et al.* 1996). Since fruit set, germination, and survival were binomially distributed and could not be log transformed, we used a heterogeneity *G*-test to evaluate the significance of the interaction between population and cross type (Sokal & Rohlf 1981; Johnston & Schoen 1994). We interpreted a significant interaction as indicating that the direction or magnitude of the differences between cross treatment effects varies among populations. We interpreted a non-significant interaction term as indicating that *both* the direction and magnitude of the differences between treatments are statistically indistinguishable across populations.

The above tests evaluate the presence of inbreeding depression and whether the magnitude of differences between cross treatments differs among populations. To obtain a value for the magnitude of population-level inbreeding depression for fitness, we first calculated mean fitness per cross type for each population in each environment using the method of least square means in Proc MIXED. We then calculated inbreeding depression as the difference between mean ON and S fitness and divided by mean ON fitness. In cases in which S fitness was greater than ON fitness, we used mean S fitness as the divisor. We performed these calculations for each population, individually, within each experimental site.

Results

Fruit & Seed Set

Cross type had no effect on fruit ($F_{2,869} = 0.08$, $p = 0.93$) or seed set ($F_{2,609} = 1.38$, $p = 0.25$) in the field, indicating that there is no inbreeding depression for either trait (Fig. 4.1). Average fruit set, pooled across all treatments and populations, was 0.90 (± 0.306 SD), and average seed set, measured as fruit mass (see Methods), was 15.57 mg (± 9.141 SD), respectively. This translates into approximately 700 seeds per fruit. There were significant effects of both family and population on seed set ($p < 0.001$), but not for fruit set ($p > 0.05$). In neither analysis was the interaction between population and cross type significant ($p > 0.05$).

Early Life History Traits in the Greenhouse

Cross type significantly affected germination in the greenhouse ($F_{2,1701} = 16.08$, $p < 0.0001$) (Fig. 4.2A). Selfed progeny suffered an approximately 16% reduction in germination compared to ON progeny ($t = 5.35$, $p < 0.0001$) and 13% reduction compared to OF progeny ($t = 4.26$, $p <$

0.0001). Germination rates of ON and OF progeny, however, were not statistically different (t = -1.05, p = 0.55). Family accounted for significant variation in germination rates ($\chi^2 = 25.7$, p < 0.0001). Neither population (p > 0.05) nor its interaction with cross type ($G^2_{10} = 2.0940$, p = 0.99) were significant, implying that selfed progeny have lower germination rates than outcross progeny across all populations.

Cross type also significantly affected seedling emergence date ($F_{2,821} = 3.41$, p = 0.03) (Fig. 4.2B). Selfed progeny germinated an average of approximately 3 days later than ON progeny (t = -2.38, p = 0.05). No difference in average emergence date was detected between OF progeny and either ON (t = 2.07, p = 0.10) or S (t = -0.35, p = 0.94) progeny. Family significantly affected average seedling emergence date ($\chi^2 = 54.2$, p < 0.0001), but this was not the case for population or its interaction with cross type (p > 0.05).

Seedling size was significantly different among crosses ($F_{2,795} = 4.20$, p = 0.02), but differences in initial growth rates among cross types fell short of significance ($F_{2,782} = 2.78$, p = 0.06) (Figs. 2C, 2D). Self progeny were significantly smaller than OF progeny (t = 2.75, p = 0.02) and marginally smaller than ON progeny (t = 2.23, p = 0.07). OF and ON progeny, however, were approximately equal in size (t = 0.57, p = 0.84). Family significantly affected seedling size ($\chi^2 = 9.2$, p = 0.002) and seedling growth rate ($\chi^2 = 18.1$, p < 0.0001), while population identity only affected seedling size ($\chi^2 = 10.7$, p = 0.001). In neither analysis was the population by cross type interaction term significant (p > 0.05).

Common garden: Survival and Fitness

The effect of cross type on survival was strong and significant in the common garden ($F_{2,734} = 21.66$, p < 0.0001) (Fig. 4.3A). S progeny suffered a 36% reduction in survival compared to ON progeny ($t = 2.71$, $p = .02$) and a 61% reduction in survival compared to OF progeny ($t = 6.35$, $p < 0.0001$). Furthermore, ON progeny survived significantly less than OF progeny ($t = 4.11$, $p = 0.0001$). Average survival across all individuals was only about 30% after four months in the common garden plot. Neither family nor population affected survival ($p > 0.5$), and a heterogeneity G-test revealed that the effect of cross on survival did not depend on population ($G^2_{10} = 2.52$, $p = 0.99$).

A similar pattern was seen for the effect of cross type on fitness in the common garden (Fig. 4.3C). Cross type significantly affected fitness ($F_{2,408} = 17.44$, $p < 0.0001$). Selfed progeny had significantly lower fitness than ON ($t = 2.79$, $p = 0.02$) and OF progeny ($t = 5.64$, $p < 0.0001$). ON progeny had significantly reduced fitness compared to OF progeny ($t = 3.34$, $p = 0.003$). Neither population nor its interaction with cross type were significant ($p > 0.05$), but there were significant differences in fitness among families ($\chi^2 = 72.7$, $p < 0.0001$).

The magnitude of inbreeding depression for fitness was high in the common environment. Inbreeding depression over all populations was approximately 43%. This indicates that selfing events lead to a 43% reduction, on average, in the number of offspring that survive to 17 months compared to outcrossing events between individuals within a population. Compared to outcross-far (OF) events, selfing events cause an even greater fitness discount, approximately 66%. Although not significant, variation across individual population estimates of inbreeding depression for fitness was broad (Fig. 4.4). For example, population BC2 experienced about 84% inbreeding depression, whereas the magnitude of inbreeding depression in population WF was negative, indicating that self progeny from WF tend to be more fit than outcross progeny in the common garden environment.

Maternal sites: Survival and Fitness

As in the common garden, cross type significantly affected survival in the maternal sites ($F_{2,581}$ = 4.61, p = 0.01) (Fig. 4.3B). S progeny survived an average of 10% less than ON progeny (t = 2.68, p = .02) and OF progeny (t = 2.44, p = 0.04). In contrast to results from the common garden plot, survival of ON and OF progeny were equivalent across maternal sites (t = -0.11, p = 0.99). A non-significant interaction term between population and cross type indicated that these results were similar within each population (G^2_6 = 3.20, p = 0.78). Survival rates in the maternal environments were high, averaging approximately 83% (± 37% SD), and similar across populations (p > 0.05). This rate, however, did vary among families (χ^2 = 12.4, p < 0.001).

Cross type also significantly affected fitness ($F_{2,264}$ = 10.22, p < 0.0001) (Fig. 4.3D). Differences among cross types paralleled those for survival. Self progeny had significantly reduced fitness than ON (t = 3.97, p = 0.0003) and OF progeny (t = 4.36, p < 0.0001), but ON and OF progeny had similar fitness (t = 0.40, p = 0.92). Significant differences in fitness existed among families (χ^2 = 64.2, p < 0.0001) and populations (χ^2 = 4.4, p = 0.04). Consistent with results from the other analyses, the interaction between population and cross type was not significant (p > 0.05).

Across maternal sites, the magnitude of inbreeding depression was approximately 33%. This indicates that selfing events in natural populations lead to a 33% decline in fitness, on average, compared to outcrossing events between individuals within a population. This estimate did not change much when the fitness of S and OF treatments were compared. Selfing events lead to a 35% reduction in fitness compared to OF events. Variation in the magnitude of inbreeding depression among populations in the maternal environments was relatively small, ranging from approximately 31% to 39% (Fig. 4.4).

Discussion

The constancy of inbreeding depression in natural populations has been doubted (Crnokrak & Roff 1999; Keller & Waller 2002). Emphasis traditionally has been placed on variation in inbreeding depression among populations rather than its pervasiveness. As a result, our ability to make predictions about the ecological and demographic effects of inbreeding has been limited. There are, however, noticeable examples that have demonstrated comparable levels of inbreeding depression (hereafter, "ID") across populations. Eckert and Barrett (1994) found high levels of ID in ten *Decodon verticillatus* populations. Despite differences in population size, and presumably, mating histories, van Treuren *et al.* (1993b) found similar levels of ID across six populations of *Scabiosa columbaria.* Similarly, Groom and Preuninger (2000) found consistent levels of ID in central and isolated populations of *Clarkia concinna*. In this study, we examined ID across six populations of the biennial *Sabatia angularis* and found it to be strong and significant in a common garden and *in situ* plantings. Thus, the results of this study add to the growing body of evidence demonstrating that ID is a common denominator among populations and suggest that ID has great potential to affect the population dynamics of *S. angularis*.

Inbreeding Depression

The presence and magnitude of ID was variable across traits and life stages in *S. angularis*. Such variation in inbreeding depression across life stages is typical (Schemske 1983; Schoen 1983; Husband & Schemske 1996). However, in contrast to predictions from Husband and Schemske (1996) concerning the timing of inbreeding depression, ID did not occur at the earliest stages of fruit and seed set in *S. angularis*. Self and outcross pollinations were equally likely to set fruit, and seed set was statistically equivalent among pollination treatments. This result is slightly different from a previous study in a large *S. angularis* population in Illinois (Dudash 1990). In that study, Dudash (1990) demonstrated a significant difference in seed set between self-pollinations and outcross pollinations wherein pollen came from a donor 85 m away. Similar results, however, were not seen for the comparison between self-pollinations and outcross events wherein pollen came from a nearby source. This discrepancy might arise if nearby outcrossing events resulted in biparental inbreeding, which can similarly reduce fitness (Dole & Ritland 1993), or it could be indicative of mild inbreeding depression for seed production in that population. The absence of strong ID at fruit and seed development in either study, however, is not surprising for this species given its monocarpic life history. In general, monocarpic species should be under strong selective pressure to develop fruits and seeds provided they receive enough pollen.

Instead, ID in the *S. angularis* study populations occurred for germination and survival. The magnitude of ID at these stages was 16% for the former and up to 36% in the common garden environment for the latter. Results also suggest that the reduced survival of selfed offspring may be attributable to later emergence of selfed progeny and, subsequently, smaller rosette size. Given that seeds were germinated in the greenhouse, the effects of inbreeding at these stages are likely to be more severe in their natural habitat (Dudash 1990; Ramsey & Vaughton 1998). Unfortunately, we could not examine the entire life cycle in the field because the extremely small seed size of this species makes planting seeds directly into soil logistically infeasible and because results might be confounded by seed banks, if present. The smaller size of selfed offspring due to later germination and potentially lower growth rates may have left them at a disadvantage with respect to establishment and resource acquisition in both environments and contributed the their significantly reduced survival.

Results from a previous study examining genetic diversity in eight *S. angularis* populations across two generations are consistent with selection against inbred individuals in natural populations (Spigler, unpublished data). The inbreeding coefficient for adult plants across the majority of populations was positive, whereas it was negative for their progeny across all populations. Assuming a constant environment between generations, the change from an excess of homozygotes to an excess of heterozygotes suggests there is selection for heterozygous, and presumably outbred individuals between these stages.

We expected the magnitude of inbreeding depression to be greater in the maternal sites than in the common garden. However, the average magnitude of ID in the maternal environments was approximately 10% less than that in the common garden. This result stands in contrast to the majority of studies examining ID in multiple environments (e.g. Dudash 1990; Ramsey & Vaughton 1998; Hayes *et al.* 2005). For example, Dudash (1990) found that ON progeny had significantly greater fitness than self progeny in the field environment, but not in a common garden. Furthermore, in their review of inbreeding depression studies, Armbruster and Reed (2005) suggested that ID is an average 69% greater in more stressful environments. Clearly, the common garden was a more stressful environment as exhibited by greater mortality at this site, but we are not sure of its cause. Possible explanations for greater mortality and, consequently, lower fitness in the common garden might include increased stress from soil erosion during establishment in the freshly plowed soil or greater solar irradiation and associated lower water availability due to a lack of canopy cover.

Population and Family Effects

Population and family identity significantly affected several of the traits examined. This was not surprising given the differences in maternal plant height, total bud and flower number, and neighbor density, for example (Spigler, unpublished data). Similarly, populations likely differed in features that can affect plant stature and performance, such as habitat type or quality (e.g. Eisto *et al.* 2000; Vergeer *et al.* 2003).

The analyses showed that populations did not significantly differ in the magnitude of inbreeding depression for any of the traits examined. This result is important as it implies that inbreeding depression is a consistent force across *S. angularis* populations. However, there was noticeable non-significant variation in the magnitude of inbreeding depression for fitness among populations in the common garden. ID was over 80% for population BC2 in the common garden while it was negative for population WF. Negative values of inbreeding depression arise when self progeny perform better than outcross progeny and might be due, for example, to local adaptation. A separate analysis, however, revealed no statistical difference between the fitness of S and ON progeny in population WF, suggesting instead that S and ON progeny from this population perform equally well in the common garden environment (data not shown). OF progeny from population WF, however, performed 3 to 3.6 times better than ON and S progeny in the common garden environment, respectively, suggesting that low within-population genetic diversity renders ON and S pollination treatments equivalent (data not shown). Thus, differences in the magnitudes of ID for fitness across populations in the common garden environment partially may reflect differences in genetic diversity within populations. The magnitude of ID for fitness across populations in the maternal environments, however, was remarkably stable between 31-39% and underscores the regularity of ID in natural *S. angularis* populations.

Outbreeding Depression

The experimental design of this study also lent itself to addressing questions about outbreeding depression and local adaptation in *S. angularis* populations. Reduced performance of progeny from long distance mating events is typically interpreted as evidence for local adaptation. Waser and Price (1989) examined outbreeding depression in *Ipomopsis aggregata* by comparing the fitness of progeny resulting from outcross events in which pollen came from an individual 10 m away versus 100 m away. They found progeny of nearby outcross events outperformed progeny from far outcrossing events. Similar results have been found in other studies as well (e.g. Waser & Price 1994; Fischer & Matthies 1997; Fenster & Galloway 2000; Waser *et al.* 2000).

We did not find evidence of outbreeding depression in *S. angularis*, in agreement with results from Dudash (1990). We found that both types of outcross pollination yielded equally fit progeny for the earliest life history stages and for later stages in the maternal environment. In the common garden, we found the opposite scenario: outcross-far progeny performed better than outcross-near progeny. OF progeny were approximately 1.6 times as likely to survive and 1.5 times as fit as ON progeny. Taken together, these results indicate that while increased genetic diversity does not increase performance within maternal sites, it may aid *S. angularis* individuals in successful colonization of new sites.

Implications for Population Dynamics

Inbreeding depression for fitness in *S. angularis* can reach almost 40% in natural populations and averages around 33%. Such high levels of ID could significantly alter demographic parameters. Furthermore, the stages at which ID were manifest have important implications for population growth and persistence. ID for fitness mainly was due to ID for germination and survival versus earlier stages such as fruit and seed set. Using population growth projection models, Mills and Smouse (1994) illustrated that inbreeding depression could significantly impact extinction probabilities and that such probabilities are more closely tied to differences in survival rather than fecundity. Similarly, Oostermeijer (2000) concluded that reductions in reproductive success were less likely to impact the regeneration capacity of populations of *Gentiana pneumonanthe* than inbreeding depression at later life stages.

Although *S. angularis* is a primarily outcrossing species (Dudash 1987; Spigler, Chapter 3), the mating system does vary with population size (Chapter 3). Small populations can exhibit selfing rates at least as high as 40% (see Chapter 3). This means that a greater proportion of individuals in small populations could have significantly reduced fitness due to inbreeding depression. This consequence, compounded by significantly reduced fruit set in small populations (Chapter 2), could lower population growth rates, increase local extinction rates, and diminish the regeneration capacity of *S. angularis* populations.

Such dire consequences, however, could be reduced or prevented by the effects of purging (Lande & Schemske 1985; Charlesworth & Charlesworth 1987; Charlesworth *et al.* 1990) or genetic rescue through immigration of pollen or seed (Richards 2000). Depending on the fitness cost, purging may occur rapidly, and the effects of ID on population dynamics would be ephemeral, at most. Some evidence supports the role of purging in reducing ID (reviewed in Byers & Waller 1999; Crnokrak & Barrett 2002), while other studies do not (van Treuren *et al.* 1993b; Belaoussoff & Shore 1995; Willi *et al.* 2005). If purging was occurring across *S. angularis* populations, one might expect to see a positive correlation between outcrossing rate and mean ID (e.g., Holtsford & Ellstrand1990; Goodwillie & Knight 2006). A correlation between outcrossing rate and mean ID across the six populations included in this study was not significant (Spigler, unpublished data). The small number of populations, however, and similar rates of outcrossing among these populations likely limits our ability to make conclusions concerning the role of purging using this method. The estimates of inbreeding depression from this study represent only one generation of inbreeding. Continuing to examine the effects of several generations of inbreeding on fitness would help to elucidate the genetic mechanism of inbreeding depression and the likelihood of its pervasiveness through time. At least for now, the current data support the notion that inbreeding depression could cause serious demographic consequences in populations of *S. angularis* and that this possibility should not be ignored.

Conclusions

While the role of inbreeding depression in population dynamics has been doubted in the past (but see Schaal & Levin 1976; Polans & Allard 1989, e.g.), it is gaining recognition. Part of this delay has been due to a fundamental deficiency of information concerning the pervasiveness of inbreeding depression across natural populations of a given species. Instead, many researchers have focused on factors that affect variation in inbreeding depression in experimental settings. By performing pollinations in the natural habitat, our approach embraces natural variation among families and populations to ask whether inbreeding depression is still detectable and significant across wild populations. We demonstrated that significant inbreeding depression exists across multiple populations of *S. angularis* in a common garden and their maternal environment. Our results, in conjunction with another study demonstrating inbreeding depression in a population of this species over 600 miles away (Dudash 1990), strongly support the conclusion that inbreeding depression is a universally important factor for *S. angularis* and establish the potential for demographic consequences of inbreeding depression in this species. Furthermore, the comparison of the magnitude of inbreeding depression in a common garden and maternal sites highlights the importance of examining inbreeding depression in the field. Population estimates of inbreeding depression in the common garden were up to two times than maternal site estimates and could lead to unrealistic predictions about the effect of ID on population dynamics.

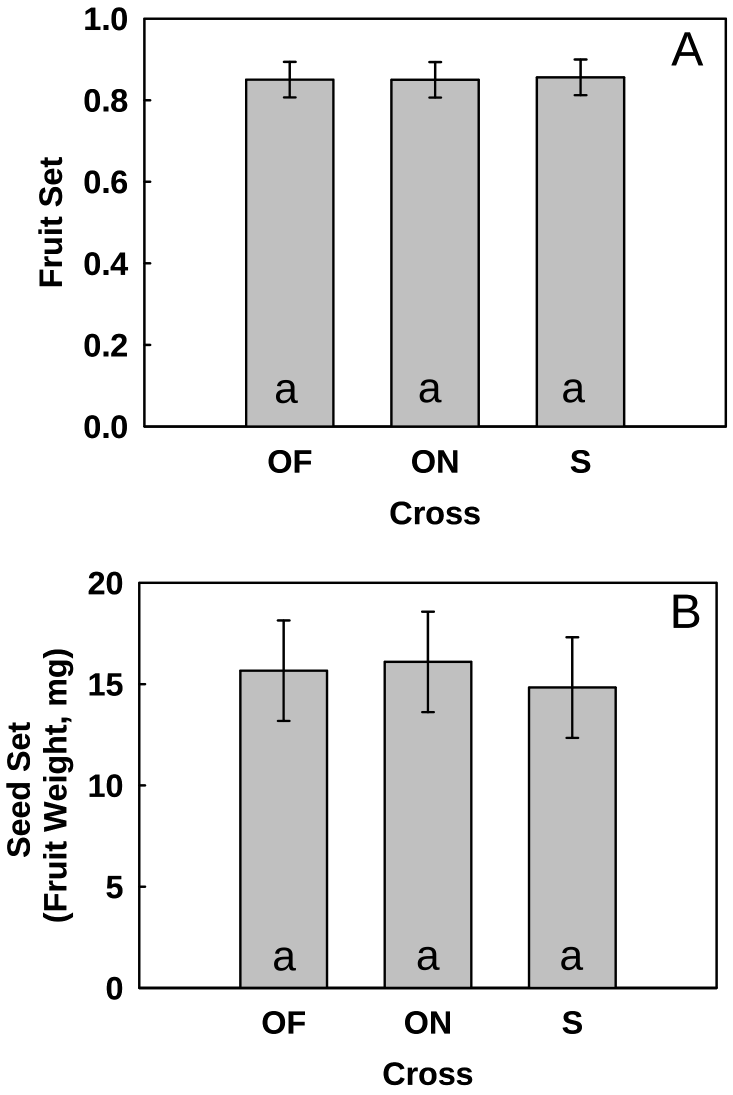

Figure 4.1. Least-squares means for fruit set (A) and seed set (B) by cross treatment. Crosses are represented as “OF” for outcross-far pollination, “ON” for outcross-near pollination, and “S” for self-pollination treatments. Different lowercase letters represent statistical differences among treatments at the $\alpha = 0.05$ level. Standard error bars are shown. Seed set was log transformed in analysis but original data are shown to allow more realistic biological interpretation.

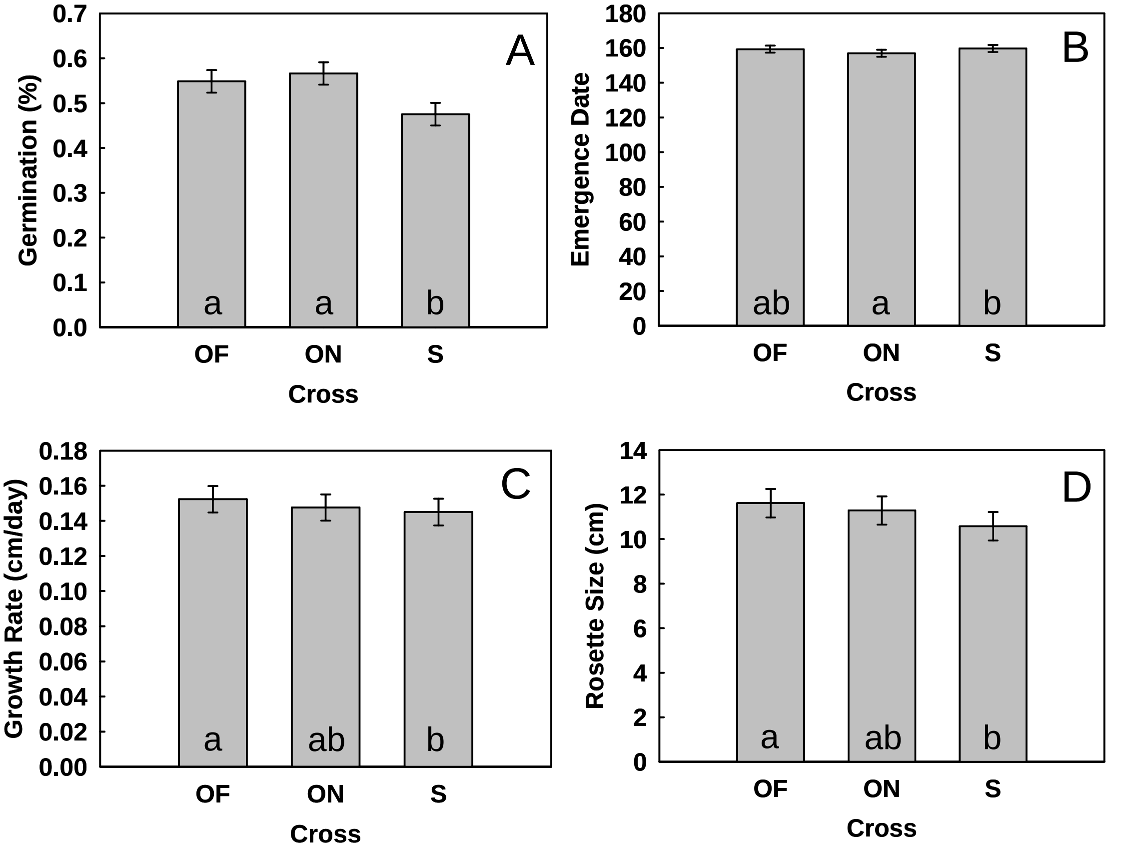

Figure 4.2. Least-squares means for (A) germination, (B) emergence date, (C), initial growth rate, and (D) rosette diameter by cross treatment. Crosses are represented as "OF" for outcross-far pollination, "ON" for outcross-near pollination, and "S" for self-pollination treatments. Different lowercase letters represent statistical differences among treatments at the $\alpha = 0.05$ level. Standard error bars are shown. Rosette size and growth rate were log transformed in analyses, but original data are plotted to allow more realistic biological interpretation.

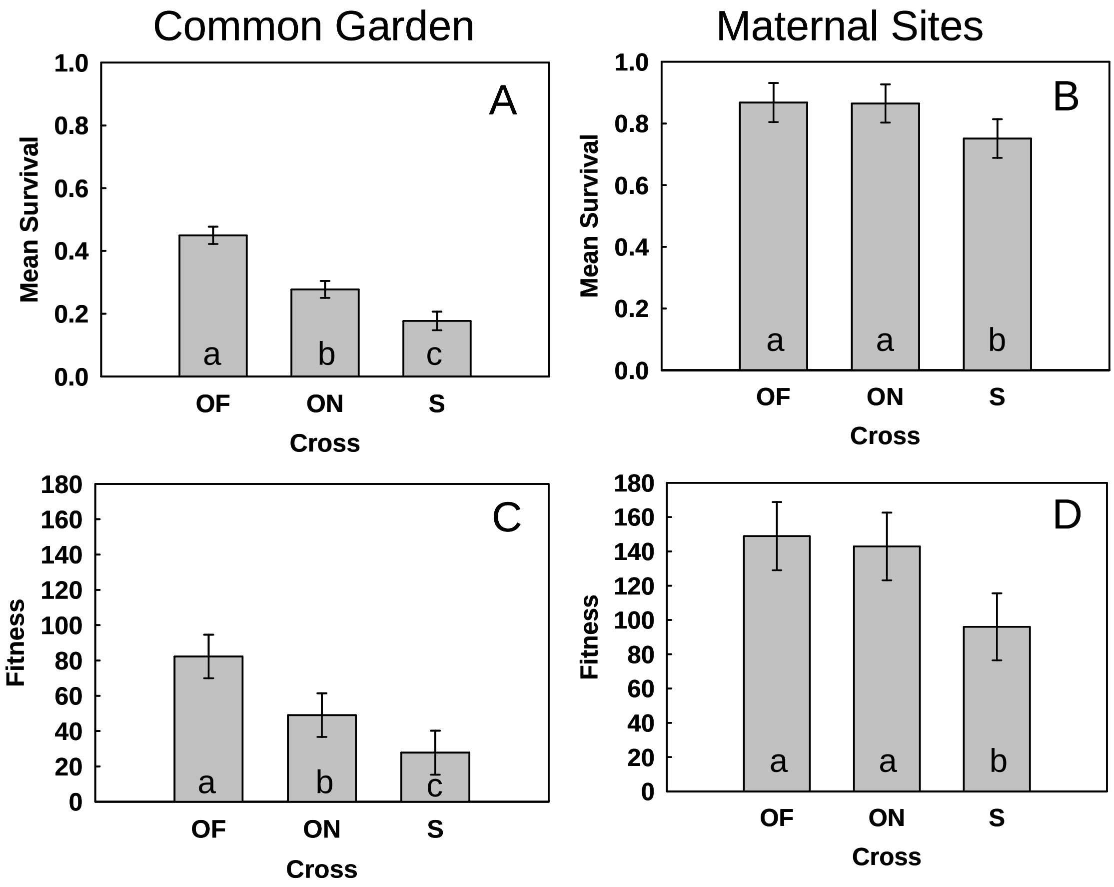

Figure 4.3. Least-squares means for (A, B) survival and (C, D) fitness in each environment by cross treatment. Crosses are represented as "OF" for outcross-far pollination, "ON" for outcross-near pollination, and "S" for self-pollination treatments. Different lowercase letters represent statistical differences among treatments at the $\alpha = 0.05$ level. Standard error bars are shown.

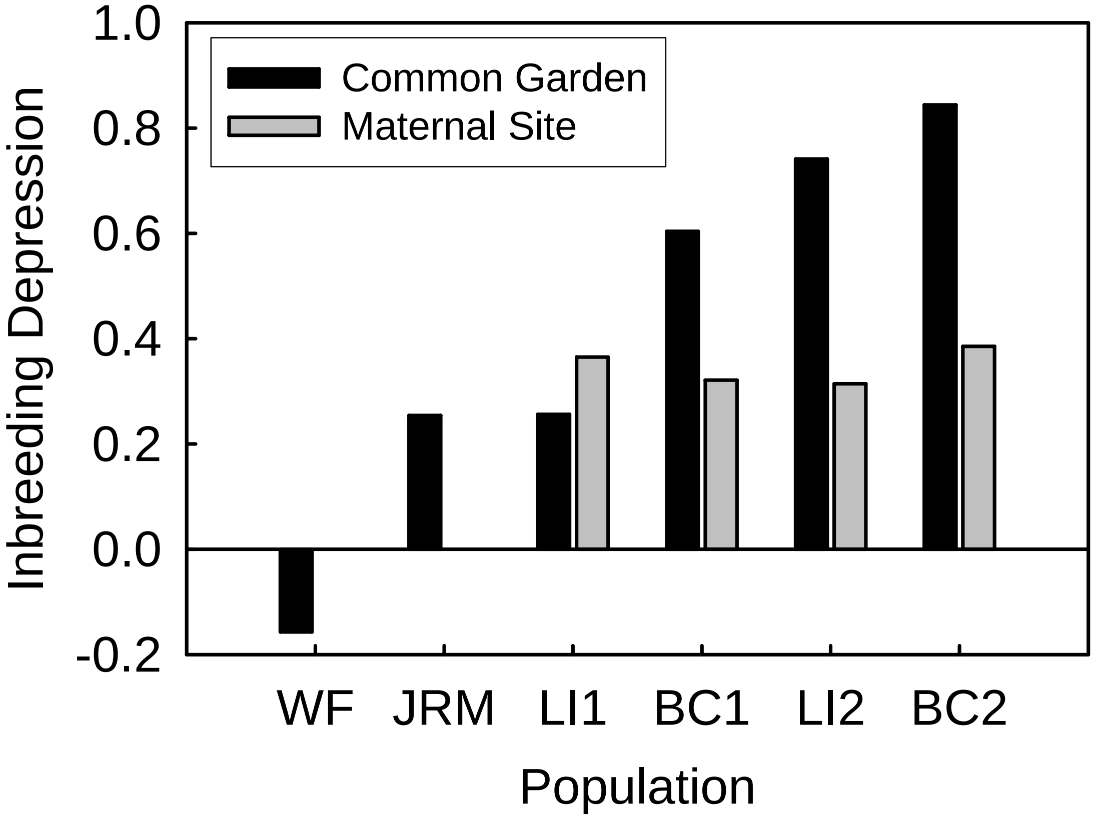

Figure 4.4. Mean inbreeding depression for fitness across the six study populations in two environments. Standard error bars are shown. Populations WF and JRM were not represented in their maternal site.

CHAPTER 5

CONCLUSIONS

Significance

Increasing habitat fragmentation and concern for rare and endangered species has motivated decades of research on the reproductive consequences of reduced population size and their implications for population persistence. This body of research suggests that small populations of these species suffer reduced reproductive success, increased selfing, decreased genetic diversity, and concomitant decreases in population fitness. Now population biologists are asking whether such trends represent general phenomena for plant species (Leimu *et al.* 2006), yet relatively less data exists on the reproductive consequences of reduced population size for natural populations of common species. Given that all populations vary in size and density, these consequences should not be restricted to species in peril. Yet our view of the dynamics of typical, small populations has been colored classically by the assumption that individuals in small populations generally should reap per capita fitness benefits due to release from competition. Studies examining the reproductive consequences of reduced population size in common species are therefore useful for evaluating this assumption and for improving our understanding of the reproductive consequences of reduced population size. Such information will provide insight into the factors that affect population persistence, exacerbate the probability of local population extinction, and constrain colonization.

In this dissertation, I examine the reproductive consequences of reduced population size in a relatively common, widespread biennial *Sabatia angularis* (Gentianaceae) to discover whether the patterns seen for rare and endangered species may be found in this common species as well. I examine how fruit set, seed set, and subsequent reproductive success vary with population size and density at population-level spatial scales and how these variables change with plant abundance on local scales. I further examine how aspects of the mating system vary with population size and density and whether small or low density populations have reduced genetic diversity. Lastly, I explore the demographic implications of inbreeding in natural populations by examining the prevalence and magnitude of inbreeding depression across multiple *S. angularis* populations.

Summary of Results

In Chapter 2, I demonstrated that population size significantly affects fruit set and may also affect reproductive success. A significant quadratic relationship was evident between population size and fruit set across all populations examined, confirming that small populations of *S. angularis* experience reductions in fruit set, but also suggesting that the largest populations suffer reproductive decreases as well. Population size alone explained almost half of the variation in mean fruit set among populations. Across all populations, there was a trend of increasing mean reproductive success with population size, suggesting decreased reproductive success in small populations. This analyses was greatly affected by the inclusion of one population, GP, which was extremely large. Most populations do not become so large, and the relationship between mean reproductive success and population size in the remaining populations revealed a significant quadratic relationship. This result parallels that seen for fruit set and again confirms that small populations of *S. angularis* suffer reproductive disadvantages. While I did not find a similar relationship between population size and pollen receipt, significantly reduced pollen receipt in population LI3, comprised of only 12 individuals, suggests that there may be a population size threshold below which pollinator visitation precipitously declines.

Furthermore, I determined that reproduction is affected by variation in plant abundance on much finer spatial scales. I demonstrated that at the very smallest spatial scale examined, increased numbers of conspecific neighbors significantly decrease seed set and subsequent reproductive success. At intermediate spatial scales, however, increases in the number of conspecific neighbors facilitates per capita seed set and reproductive success. These results highlight the importance of facilitative interactions for reproductive success and the importance of examining these interactions on multiple spatial scales.

In Chapter 3, I demonstrated a significant positive correlation between outcrossing rate and population size. This correlation was strong, and population size explained almost half of the variation in outcrossing rate. While mean selfing rate across populations was 22%, this value almost doubled in the smallest population to 40% and was only about 7% in the very largest. I also showed that biparental inbreeding tends to increase with population density. This may suggest greater spatial genetic structuring in dense populations and may be a consequence of limited seed dispersal. Despite finding a significant relationship between the outcrossing rate and population size, I did not see similar patterns between population size or density and standard genetic diversity statistics. Instead, I demonstrated that heterozygosity increased from the seedling to adult stage across all eight populations examined and that the number of alleles per locus tended to decline across those stages in most populations. I suggest that these patterns are due to selection for heterozygotes, and presumably outbred, progeny.

In Chapter 4, I determined significant inbreeding depression in multiple *S. angularis* populations in a common garden environment and in natural populations. I found that significant inbreeding depression occurred for germination, rosette size, and survival, which culminated into significantly reduced fitness of inbred offspring; selfing events in natural populations led to an approximately 33% decline in the subsequent number of offspring surviving to 17 months than outcrossing events between individuals within populations. This decline may have significant implications for population growth, particularly considering that these effects are manifest during germination and survival, stages that are more likely to affect population dynamics (Mills & Smouse 1994; Oostermeijer 2000).

Conclusions and Future Directions

The main conclusion of this dissertation is that common species are not impervious to the consequences of reduced population size. Small populations of *S. angularis* experienced significantly reduced fruit set and reproductive success and significantly increased selfing rates. These results support the emerging viewpoint that such consequences are a general phenomenon for plant species. Furthermore, these consequences may have severe implications for the persistence of small *S. angularis* populations.

While some studies have shown increased recruitment with increased seed production (Ackerman *et al.* 1996; Lennartsson 2002), reductions in fecundity alone may be less likely to impact population dynamics unless recruitment is seed limited. Populations of *S. angularis* seem unlikely to be seed limited given that I found a given plant can produce almost 150,000 seeds alone. Instead, small *S. angularis* populations may be more likely to face problems posed by increases in the selfing rate. Selfing rates in small populations can be at least as high as 40%. Given that selfed progeny experience significantly reduced germination and survival, higher selfing rates in small populations translates into a greater proportion of progeny suffering significant fitness declines. If high levels of selfing persist in small populations, significant decreases in germination and survival could result in decreased population growth rates, unless there are large seed banks, which could buffer these effects (e.g., Baskin & Baskin 1978). These effects could become exacerbated by decreased reproduction of inbred offspring (Dudash 1990).

I will continue following study individuals from Chapter 4 to discover whether inbred individuals from the study populations experience significantly reduced reproductive success as well.

On the other hand, selection against inbred individuals may be so strong that the negative effects of inbreeding depression are purged quickly from populations, negating or limiting the effects of inbreeding on population growth rates. Data from Chapter 3 showed a trend for positive inbreeding coefficients in the progeny generation, but mostly negative inbreeding coefficients in the maternal generation. This trend suggests that there is viability selection against homozygous, and presumably inbred, individuals between the seedling to adult stage, consistent with the experimental results from Chapter 4. Thus, the ultimate effects of inbreeding on population dynamics will depend on a balance between inbreeding depression and selection against inbred individuals.

Continuing these studies over multiple generations in natural populations would elucidate the consistency of the reproductive consequences of reduced population size in *S. angularis* and their subsequent consequences for population persistence, over time. In addition to these long term studies, modeling exercises including information on the relationships between population size, reproductive success, and the selfing rate and their effects on demographic transitions would greatly aid our ability to predict the long term consequences of the reproductive consequences of reduced population size for *Sabatia angularis.*

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APPENDIX A

ALLELE FREQUENCIES FOR EIGHT POLYMORPHIC LOCI ACROSS NINE *SABATIA ANGULARIS* POPULATIONS

APPENDIX A. Allele frequencies for eight loci across nine *Sabatia angularis* populations. Note: GP was not included in the final analysis of the relationship between population size and mating system parameters because of its extrememly large size.

		POPULATION								
LOCUS	**ALLELE**	**BC1**	**BC2**	**WF**	**COW2**	**LI1**	**LI2**	**JRM**	**GP**	**WOM**
DIA1	3	0	0.014 (0.009)	0.010 (0.007)	0.009 (0.011)	0.021 (0.011)	0.023 (0.012)	0.002 (0.002)	0.004 (0.004)	0.711 (0.055)
	4	0	0.986 (0.009)	0.035 (0.011)	0.040 (0.028)	0	0.047 (0.016)	0.002 (0.002)	0.006 (0.005)	0.152 (0.045)
	5	1.000 (0)	0	0.955 (0.013)	0.951 (0.035)	0.979 (0.011)	0.929 (0.022)	0.996 (0.003)	0.990 (0.006)	0.136 (0.039)
DIA2	3	0	0	0.010 (0.008)	0.009 (0.009)	0.687 (0.056)	0.671 (0.108)	0.014 (0.010)	0.026 (0.013)	0.006 (0.005)
	4	1.000 (0)	1.000 (0.000)	0.990 (0.008)	0.991 (0.009)	0.313 (0.056)	0.329 (0.108)	0.986 (0.010)	0.974 (0.013)	0.994 (0.005)
FE1	3	0.007 (0.007)	0	0.019 (0.009)	0.561 (0.099)	0.109 (0.099)	0.785	0.405 (0.109)	0.230 (0.045)	0.448 (0.042)
	4	0.993 (0.007)	1.000 (0.000)	0.981 (0.009)	0.433 (0.099)	0.891 (0.099)	0.215	0.595 (0.109)	0.761 (0.043)	0.552 (0.042)
	5	0	0	0	0	0	0	0	0.009 (0.006)	0
FE2	3	0.389 (0.066)	0.166 (0.047)	0.237 (0.027)	0.096 (0.077)	0.051 (0.012)	0.182 (0.051)	0.218 (0.047)	0.299 (0.046)	0.384 (0.094)
	4	0.611 (0.066)	0.834 (0.047)	0.761 (0.027)	0.904 (0.077)	0.641 (0.075)	0.720 (0.059)	0.753 (0.050)	0.701 (0.046)	0.610 (0.093)
	5	0	0	0	0	0.278 (0.073)	0.025 (0.017)	0.021 (0.020)	0	0.006 (0.005)
	6	0	0	0.002 (0.002)	0	0.031 (0.016)	0.073 (0.037)	0.009 (0.009)	0	0
FE4	4	0.958 (0.013)	0.899 (0.036)	0.672 (0.047)	0.252 (0.082)	0.969 (0.016)	0.968 (0.012)	0.977 (0.012)	0.977 (0.011)	0.946 (0.017)
	5	0.042 (0.013)	0.101 (0.036)	0.328 (0.047)	0.748 (0.082)	0.031 (0.016)	0.032 (0.012)	0.023 (0.012)	0.023 (0.011)	0.054 (0.017)
6PGD3	3	0.889 (0.027)	0.851 (0.029)	0.053 (0.016)	0.030 (0.016)	0.007 (0.006)	0.695 (0.058)	0.780 (0.068)	0.055 (0.016)	0.038 (0.018)
	4	0.111 (0.027)	0.149 (0.029)	0.947 (0.016)	0.970 (0.016)	0.993 (0.006)	0.305 (0.058)	0.220 (0.068)	0.945 (0.016)	0.962 (0.018)
UGPP3	3	0	0.006 (0.006)	0	0.050 (0.024)	0.002 (0.002)	0	0	0.024 (0.014)	0.032 (0.012)
	4	0.990 (0.007)	0.988 (0.008)	0.988 (0.006)	0.937 (0.022)	0.818 (0.023)	0.612 (0.077)	0.921 (0.032)	0.894 (0.022)	0.964 (0.013)
	5	0.010 (0.007)	0.006 (0.006)	0.012 (0.006)	0.013 (0.092)	0.180 (0.023)	0.388 (0.077)	0.079 (0.032)	0.082 (0.021)	0.004 (0.004)
ME2	1	0.053 (0.021)	0.047 (0.027)	0.395 (0.069)	0.286 (0.090)	0.113 (0.031)	0.085 (0.044)	0.206 (0.054)	0.225 (0.056)	0.155 (0.039)
	2	0.356 (0.077)	0.174 (0.056)	0.060 (0.021)	0.027 (0.016)	0.238 (0.055)	0.093 (0.034)	0.197 (0.049)	0.203 (0.048)	0.035 (0.012)
	3	0.515 (0.068)	0.779 (0.058)	0.545 (0.074)	0.687 (0.092)	0.649 (0.066)	0.821 (0.066)	0.597 (0.071)	0.572 (0.054)	0.809 (0.042)
	4	0.077 (0.035)	0	0	0	0	0	0	0	0