

Effects of plant abundance on reproductive success in the biennial *Sabatia angularis* (Gentianaceae): spatial scale matters

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Summary

1. Small and low-density populations often suffer significant reductions in reproduction, as exemplified by studies on rare and threatened species. While this phenomenon is less studied in common species, if general, it should not be restricted to those in peril. We addressed this assertion by examining the effects of plant abundance, measured at population-level (population size, density) and local-level (local neighbourhood size, LNS) spatial scales, on fruit set, seed set and subsequent reproductive success (RS) across 19 natural populations of the widespread biennial *Sabatia angularis* (Gentianaceae). We also examined stigmatic pollen loads across a subset of populations to address whether changes in reproduction were related to reduced pollination.

2. Effects of plant abundance on reproduction were evident at both spatial scales. At the population level, population size – but not density – affected fruit set significantly. This effect was non-linear. Fruit set in populations larger than 15 individuals was relatively constant, but dropped abruptly in populations below that size. This relationship did not prevail between population size and RS. Instead, RS declined significantly with population density.

3. At the local level, increases in LNS between 1-m and 4-m radii from study plants increased fruit set significantly. In contrast, increases within 1 m significantly reduced seed set. Both of these effects prevailed at different spatial scales for RS; increases in 1-m LNS reduced RS, but increases beyond that distance and within 4 m increased RS.

4. Although non-significant, trends in the data are consistent with facilitative interactions for pollination influencing local-level effects on fruit set. However, negative interactions, presumably because of competition for resources, are more important for determining seed set at local scales. Both interactions influence RS, but the effect of competition appears to dominate at the population level.

5. Synthesis. This study demonstrates that although significant reductions in fruit set in small *S. angularis* populations imply that common species are not impervious to reproductive disadvantages, small populations need not experience reduced RS. Furthermore, low-density populations actually enjoy increased RS. As such, this study highlights the complexity of ecological interactions affecting reproduction and the importance of incorporating multiple spatial scales when examining population dynamics.

Key-words: competition, density, facilitation, fruit set, local neighbourhood size, pollen load, population size, reproductive success, *Sabatia angularis*, seed set

Introduction

Evidence accumulating over the past decade demonstrates that plants in small and/or low-density populations often suffer decreases in reproductive success (RS) (Lamont *et al.*

1993; Widén 1993; Roll *et al.* 1997; Groom 1998; Kéry *et al.* 2000; 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 cannot compete successfully for pollinators against more

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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; Kalisz *et al.* 2004). However, despite alleviating pollen limitation when pollinators are scarce, autogamy can still result in lower fruit set if inbred fruits are aborted selectively (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, also affecting RS. Often this is mediated through changes in pollinator behaviour. Pollinators commonly increase intra-plant movements relative to inter-plant movements when plants are at low density (Antonovics & Levin 1980; van Treuren *et al.* 1993; 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 (Waite & 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 behaviour 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 prove 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 reproductive consequences of reduced population size and density have been conducted solely on population-level spatial scales, and often only estimates of crude density are reported. Yet plants rarely are distributed uniformly within populations. As a result, measures of abundance on large scales often do not represent crowding in a plant's local neighbourhood accurately. The distinction between crude density and crowding in a local neighbourhood may be important because ecological interactions affecting RS may be described better on small spatial scales. For example, the effects of resource competition among individuals are best characterized on local spatial scales because resources are often localized and plants are sessile (Mack & Harper 1977; Pacala & Silander 1985; Silander & Pacala 1985). In addition, many pollinators behave in ways that optimize foraging (Pyke *et al.* 1977; Pyke 1978) and thus may be more responsive 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 the effects of plant abundance on RS on local scales (Allison 1990; House 1992; Widén 1993; Roll *et al.* 1997; Ghazoul *et al.* 1998; Mitchell & Ankeny 2001), studies examining these effects on both local- and population-level spatial scales are rare (but see Wagenius 2006; Gunton & Kunin 2007).

In this study, we evaluate the effect of plant abundance on reproduction in natural populations of the widespread, native biennial *Sabatia angularis* (L.) Pursh (Gentianaceae) on two spatial scales. We ask the following questions. (i) At the population level, do mean population fruit set, seed set and subsequent reproductive success increase with population size or population density? (ii) At the local level, do per-capita fruit set, seed set and reproductive success increase with local neighbourhood size (LNS), defined as the number of neighbouring conspecifics within 1-m, 2-m and 4-m radii surrounding focal

Table 1. Summary data for study populations including location, population size, population density and average local neighbourhood size.

Population	Abbreviation	Location	Population size	Density	Average local neighbourhood size (SD)	
					$r = 1$ m	$r = 4$ m
Buck Creek Extension	BCE	Clay Co., NC	1	NA	0	0
Buck Creek Trail (middle)	BCTM	Clay Co., NC	1	NA	0	0
Buck Creek Trail (end)	BCTE	Clay Co., NC	2	NA	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)

SD, standard deviation; NA, not applicable; NC, North Carolina; SC, South Carolina; GA, Georgia.

study plants? (iii) How does the response of reproduction to plant abundance compare across spatial scales?

We hypothesize that higher visitation rates in large or dense populations and pollinator facilitation in larger local neighbourhoods will lead to increased reproduction. We test these hypotheses by examining whether (i) fruit and seed set increase with stigmatic pollen load, (ii) relationships exist between mean stigmatic pollen load per population and either population size or density, and (iii) stigmatic pollen load per individual increases with LNS.

Methods

STUDY SPECIES AND SITES

Sabatia angularis is an herbaceous biennial native to the eastern United States and south-eastern Canada. It is widely distributed throughout its range and is found in a variety of habitats including glades, marshes, rocky outcrops, old fields, roadsides, prairies and serpentine barrens (USGS 2003). *Sabatia 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 4 days (Dudash 1987), but can last over a week if unpollinated (R. Spigler, pers. obs.). Although flowers are protandrous, individuals produce flowers continuously during their flowering period; consequently, they 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 offer pollen as a reward to a generalist suite of pollinators that includes leaf-cutter bees (Megachilidae), sweat bees (Halictidae), andrenid bees (Andrenidae), small carpenter bees (Anthophoridae) and hover flies (Syrphidae)

(Dudash 1987; R. 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 to July 2004, we identified 19 *S. angularis* populations in Georgia, North Carolina and South Carolina (Table 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 kilometres separated most study populations. Because natural *S. angularis* populations are patchy and discrete, estimates of local population size are feasible and accurate. We assessed population size by counting all reproductive individuals. Populations ranged in size from only one individual to 355 individuals (Table 1).

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 per m² among study populations and was independent of population size ($\rho_{16} = 0.41$, $P = 0.12$). Within each population, we tagged and mapped all individuals to the nearest centimetre. We used this map information to calculate the number of conspecific neighbours within 1-m, 2-m and 4-m radii surrounding focal individuals (hereafter referred to as LNS). We included multiple radii because 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 study populations with more than two individuals were 0.57–19.5 (1-m LNS), 1.37–44.5 (2-m LNS) and 2.4–80.5 (4-m LNS) (Table 1).

ESTIMATING FRUIT SET, SEED SET AND 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, measured their height and monitored them continuously until fruit maturation. Prior to flowering, we counted the number of buds per focal plant. We estimated per-capita fruit set as the ratio of fruit number to total bud number per plant. We randomly collected up to 20 fruits per focal plant after ripening but prior to dehiscence in order 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 0.022 ± 0.001 SE mg seed⁻¹) (R. Spigler, unpubl. 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 relationship between seed number and seed mass ($F_1 = 568.4$, $P < 0.0001$, $R^2 = 0.98$) (R. Spigler, unpubl. data). Conventionally, reproductive success is measured as the total number of seeds produced per plant. We calculated reproductive success by first converting our proxy for seed set into average seed number per fruit using a regression relating seed mass to seed number (R. Spigler, unpubl. data). 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 stigmatic pollen loads in the following eight populations, which represented a range of population sizes and population densities: LI3, JRM, LI1, BC3, CFR, WF, DP and WOM (in order of smallest to largest) (see Table 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. However, in population LI3, we had to collect stigmas from focal plants because the population consisted of only 12 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 air-tight 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 at random and counted pollen grains on the top quarter of that 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 and reproductive success

To test the predictions that mean fruit set, seed set and subsequent reproductive success increase with population size or population density, we first calculated population means for each response variable. Specifically, we calculated least-squares means, which we obtained by modelling each trait of interest as a function of population identity and plant height in PROC GLM and by using the LSMEANS statement (SAS 1999). The method of least-squares adjusts arithmetic means so that they are independent of variation

in specified covariates. We included plant height as a covariate because it is known to significantly affect reproduction in this species (R. Spigler, unpubl. data). Plants in this study ranged in height from 2 cm to 102 cm, and mean plant height per population ranged from 16.5 cm in population BCTE to 62.6 cm in population HIA. This variation in plant height among study populations is significant ($F_1 = 11.96$, $P < 0.0001$), but is unrelated to variation in population size ($F_1 = 0.56$, $P = 0.47$) and density (log-transformed, $F_1 = 0.42$, $P = 0.53$). Thus, the use of least-squares means allows us to evaluate the effects of population size and density on reproduction independently of plant height.

We used regression analysis in PROC GLM (SAS 1999) to evaluate the effects of population size and density on mean fruit set, seed set, and reproductive success. Because the density of population WOM is 2.4 times as great as that of the second most dense population (see Table 1), we transformed population density using the natural logarithm to decrease the leverage of this population in influencing the evaluated relationships. In addition, population BCE had extremely high fruit (0.97) and seed set (12.25 mg fruit⁻¹). Regression diagnostics revealed population BCE as an outlier in the fruit set analyses. Because it also represents a biological reality, we present results from analyses with and without this population included.

Scatter plots of mean fruit set and mean reproductive success against population size suggested non-linear relationships (Fig. 1). Therefore, we evaluated these relationships further with a quadratic model. A quadratic function might be appropriate if increased competition, for instance, reduced fruit set or reproductive success in the largest populations. However, one expects *a priori* that reproduction should not increase infinitely with population size. This is particularly true for fruit set, which can never be greater than one. Therefore, a function in which fruit set begins to saturate beyond some population size may be more appropriate given the data (e.g. Kunin 1993). We fit the fruit set data to the following saturation (negative logistic) function using PROC NLIN in SAS (1999): $M \times [1 - 1/(1 + \exp(s \times x))]$ (adapted from Kunin 1993). The variable M represents the asymptotic maximum value for fruit set, and s describes the rate at which fruit set initially increases with x , which represents population size. We calculated approximate R^2 values for this function using $1 - (SSE/SST_{corrected})$, where SSE is the error sum of squares and SST is the total sum of squares. We did not fit a saturation curve to the reproductive success data because it did not appear appropriate from the scatter plot.

We analysed the effect of LNS on individual fruit set, seed set and reproductive success using mixed multiple regression models. In contrast to the population-level analyses, in which we use population mean trait values, these analyses are based on individual measures. We included individual-based 1-m and 4-m LNS as fixed effects and population identity as a random effect to account for variation attributable to differences among populations. We removed 2-m LNS from the analyses because of high correlation with both 1-m LNS ($\rho_{359} = 0.81$, $P < 0.0001$) and 4-m LNS ($\rho_{359} = 0.87$, $P < 0.0001$). In all analyses that included LNS, we treated 4-m LNS as the number of additional individuals beyond 1-m LNS within 4 m to increase the independence of these factors ($\rho_{359} = 0.39$, $P < 0.0001$). Almost 30% of individuals across 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 1-m and 4-m LNS on seed set (square-root-transformed)

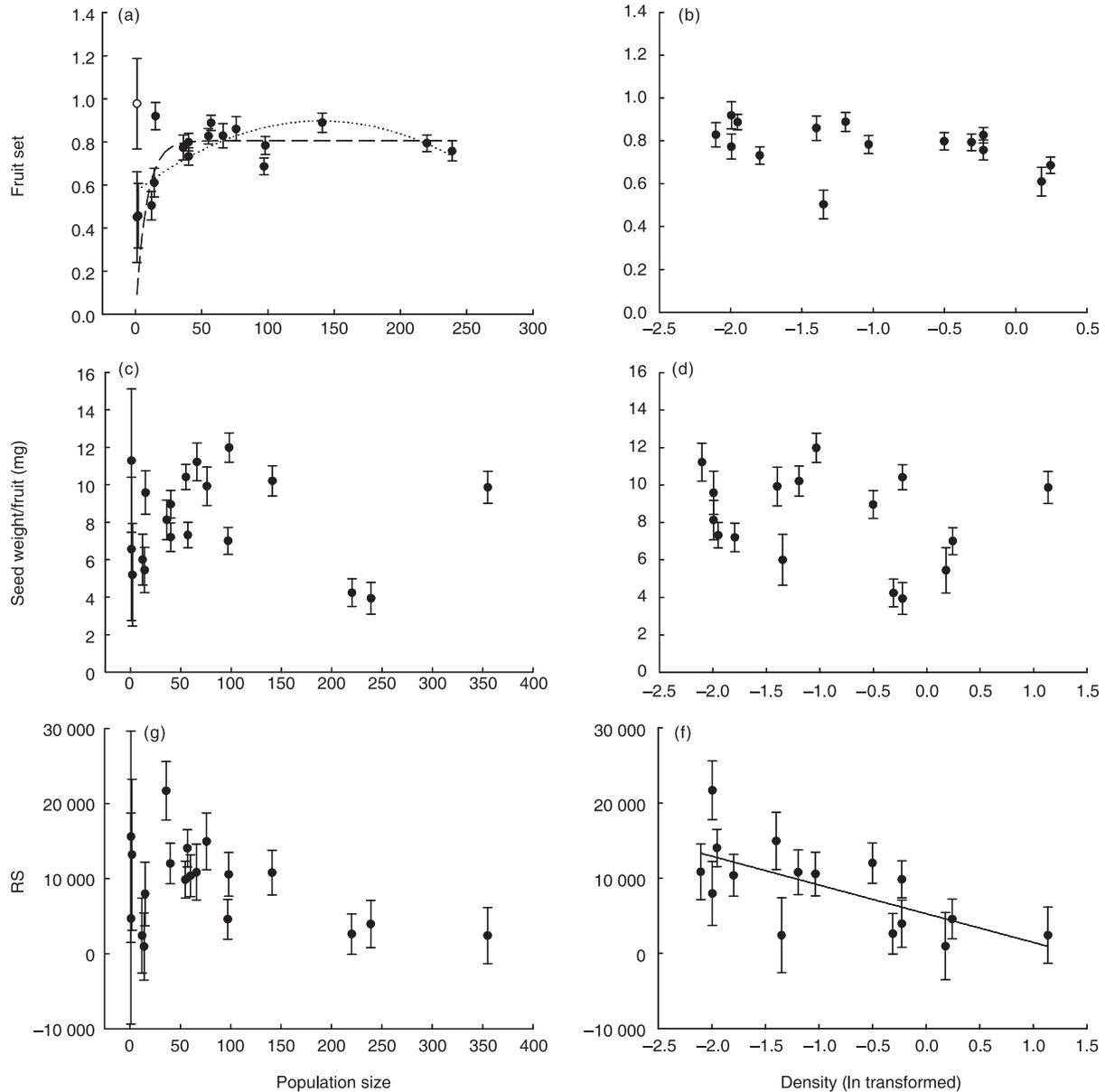


Fig. 1. Relationships between population size and (a) mean fruit set, (c) mean seed set (measured as mean total seed weight fruit⁻¹ in mg) and (e) mean reproductive success (RS), and between density and (b) mean fruit set, (d) mean seed set and (f) mean RS. All mean values are least-squares means (see Methods). Regression lines are drawn if the relationship is significant. The broken line in (a) represents the saturation model and the dotted line represents the quadratic model. The open circle in (a) represents population BCE, which is not included in the presented models. Standard error bars are shown for population least-squares means.

and RS (log-transformed). We included plant height as a fixed covariate in all analyses. We used the natural logarithm of 1-m and 4-m LNS to decrease the high skew of these variables. Interactions between fixed effects were also examined. We removed non-significant interaction terms from the model, and re-ran the analysis 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 assess the significance of population identity as a random variable.

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. 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.

Pollen load

We used regression analyses to evaluate the effect of population size and population density (natural log-transformed) on mean stigmatic

Table 2. Effects of population size and population density on mean population fruit set, seed set and reproductive success. Population density was log-transformed. R^2 values are adjusted for the quadratic models

	d.f.	<i>F</i>	<i>P</i>	Model R^2
Fruit set*				
Population size				
Linear model	1	2.76	0.118	0.16
Quadratic model†	2	5.95	0.014	0.46
Population size	1	10.74	0.006	
Population size × population size	1	7.88	0.014	
Saturation model†	2	598.83	<0.0001	0.90
Population density	1	1.41	0.259	0.10
Seed set				
Population size	1	0.10	0.452	0.01
Population density	1	0.58	0.458	0.04
Reproductive success				
Population size				
Linear model	1	1.43	0.249	0.08
Quadratic model	2	1.87	0.188	0.20
Population size	1	1.06	0.319	
Population size × population size	1	2.21	0.158	
Population density	1	9.86	0.007	0.41

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

†Results presented exclude population BCE.

pollen load per population in the subset of eight populations. To examine if positive relationships also occurred on an individual-based scale with respect to local neighbourhood size, we used a mixed-model approach. We included individual pollen load per stigma as the dependent variable, 1-m and 4-m LNS (log-transformed) as fixed effects and population identity 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 analyses to examine the effect of mean population pollen load on mean population fruit and seed set. Least-squares means for fruit and seed set were used for consistency. We could not address these at the individual level because 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 were significant, non-linear relationships between population size and mean population fruit set (Fig. 1; Table 2). Both the saturation and quadratic models explained significant variation in mean fruit set among populations. The saturation model explained 90% of the variation in mean fruit set among populations when we excluded population BCE as an outlier. The estimated maximum asymptotic value for fruit set from this model was 0.81 (± 0.03 SE) (M) and the slope parameter (s) was 0.12 (± 0.04 SE). The quadratic model was also significant and accounted for approximately 46% of the variation in mean fruit set when population BCE was excluded (Table 2). When population BCE was included in these analyses, the quadratic model was no longer significant ($F_2 = 1.92$, $P = 0.1809$). The saturation

model remained significant ($F_2 = 195.03$, $P < 0.0001$, $M = 0.82 \pm 0.05$, $s = 0.13 \pm 0.07$), although its explanatory power was reduced to 67%. In contrast to population size, population density was not related to mean fruit set (Fig. 1; Table 2). Overall, least-squares mean fruit set across populations was 0.75 (± 0.04 SE) and ranged from 0.45 (± 0.21 SE) in BCTM to 0.98 (± 0.21 SE) in BCE.

At the local level, individual fruit set increased with local neighbourhood size at both 1-m and 4-m scales (1-m LNS slope = 0.207 ± 0.090 SE; 4-m LNS slope = 0.453 ± 0.214 SE) (Table 3). As expected, plant height also affected fruit set significantly. There was a significant interaction between plant height and 4-m LNS (slope = -0.010 ± 0.003 SE). No other interaction terms were significant ($P > 0.05$). Population identity also explained significant variation in fruit set ($\chi^2_1 = 37.7$, $P < 0.0001$).

SEED SET

Neither population size nor density explained variation in mean population seed set, measured as mean total seed mass per fruit per population (Fig. 1; Table 2). Least-squares mean seed set across populations was 8.13 mg (± 0.57 SE), which translates into approximately 370 seeds per fruit. Across populations, least-squares mean seed set varied from 3.94 mg ($\pm 0.0.86$ SE) (approx. 179 seeds fruit⁻¹) in DP (239 plants) to 11.98 mg (± 0.78 SE) (approx. 545 seeds fruit⁻¹) in CFR, a mid-sized population (98 plants).

The individual-based analysis for the effect of local neighbourhood size on individual seed set across populations revealed that 1-m LNS and plant height affected seed set significantly (Table 3). There was a significant, negative effect of 1-m LNS on seed set (slope = -0.101 ± 0.051 SE). As

Table 3. Effects of local neighbourhood size (LNS) on individual fruit set, seed set, reproductive success and stigmatic pollen load. LNS within 4 m represents the number of additional individuals between a 1 m radius and 4 m radius. LNS and reproductive success were log-transformed and seed set and pollen load were square-root-transformed. Individuals from WOM were not included in the fruit set analysis. Interaction terms are listed if significant

	d.f.	<i>F</i>	<i>P</i>
Fruit set			
1-m LNS	1, 327	5.31	0.022
4-m LNS	1, 318	4.49	0.035
Plant height (cm)	1, 328	8.59	0.004
4-m LNS × plant height	1, 328	8.92	0.003
Seed set			
1-m LNS	1, 334	3.86	0.050
4-m LNS	1, 220	1.62	0.204
Plant height (cm)	1, 336	52.62	<0.0001
Reproductive success			
1-m LNS	1, 310	4.72	0.031
4-m LNS	1, 319	11.46	0.001
Plant height (cm)	1, 324	81.80	<0.0001
4-m LNS × plant height	1, 327	6.05	0.014
Pollen load			
1-m LNS	1, 94.6	0.97	0.327
4-m LNS	1, 46.3	3.73	0.060

expected, seed set increased with plant height. However, the number of neighbours within 4 m did not affect seed set, nor did any of the interaction terms ($P > 0.05$). Mean seed set per plant ranged considerably across individuals, from only 0.50 mg fruit⁻¹ (approx. 23 seeds) for an individual in population BC3 to 24.59 mg (± 0.95 SE) fruit⁻¹ (approx. 1118 seeds) for an individual in population CFR. Population identity also explained a significant portion of this variation in seed set ($\chi^2 = 81.4$, $P < 0.0001$).

REPRODUCTIVE SUCCESS

Mean reproductive success declined significantly with increasing population density (Fig. 1; Table 2). In contrast, neither a linear nor a quadratic relationship between population size and mean reproductive success was significant, regardless of whether BCE was included in the data set. However, a negative relationship between population size and mean reproductive success was significant among populations with greater than 30 individuals ($F_1 = 14.74$, $P = 0.003$, $R^2 = 0.57$). Least-squares means for reproductive success ranged from approximately 971 seeds plant⁻¹ in population HWY to approximately 21 688 seeds plant⁻¹ in population HIA. Mean reproductive success across populations was approximately 9134 (± 1280 SE) seeds plant⁻¹.

The individual-based analysis revealed that both 1-m LNS and 4-m LNS had significant, but opposing, effects on reproductive success (Table 3). Increasing 1-m LNS had a negative effect on per-capita reproductive success (slope = -0.076 ± 0.035 SE), while 4-m LNS had a positive effect (slope = 0.234 ± 0.069 SE). Height and its interaction with 4-m LNS were

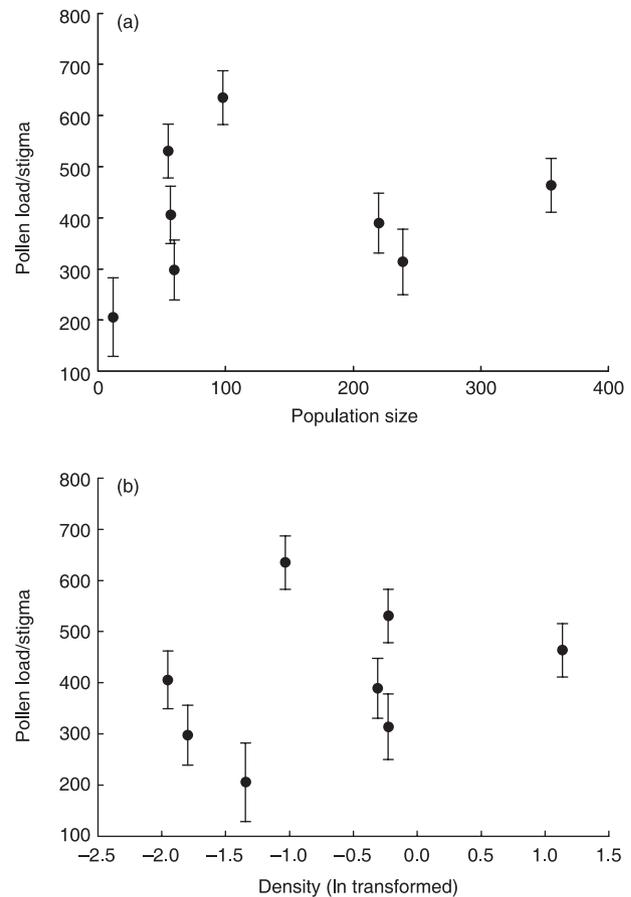


Fig. 2. Relationships between mean stigmatic pollen load per population and (a) population size and (b) population density. Standard error bars are shown.

also significant (Table 3), as was population identity ($\chi^2 = 40.4$, $P < 0.0001$). Across individuals, the range in reproductive success was large. 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.

POLLEN LOAD

At the population level, neither population size ($F_1 = 0.12$, $P = 0.747$) nor population density ($F_1 = 0.31$, $P = 0.595$) significantly predicted mean stigmatic pollen load per population (Fig. 2). Across populations, mean stigmatic pollen load was approximately 405 (± 48 SE) grains. Population CFR received the most pollen grains per stigma sample (approximately 634 grains ± 50 SE), on average, and population LI3 received the least amount (approx 206 grains ± 37 SE).

At the local level, neither LNS significantly predicted individual pollen load (Table 3). There was significant variation in pollen load among populations ($\chi^2 = 11.5$, $P < 0.001$). Although not significant, there was a trend toward a positive effect of 4-m LNS on pollen load, suggesting that larger neighbourhood sizes at this spatial scale may increase pollen

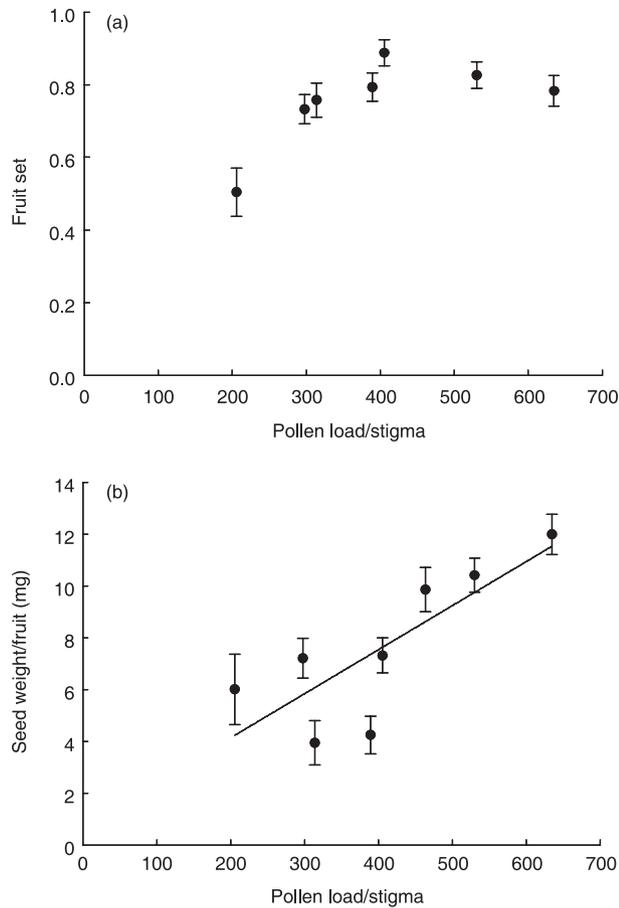


Fig. 3. Relationships between mean stigmatic pollen load and (a) least-squares mean fruit set and (b) least-squares mean seed set across eight study populations. Note only seven populations are included in (a) because WOM was not included in fruit-set analyses. Standard error bars for least-squares means are shown. Regression lines are drawn if the relationship is significant.

load (slope = 1.294 ± 0.670 SE) (Table 3). Individuals varied greatly in pollen load from only 58 grains on a stigma sample from a plant in population LI3 to 1186 grains on a stigma sample from a plant from population JRM. We rarely found heterospecific pollen on the collected stigmas, and therefore excluded its potential influence on reproductive success in these populations.

We also examined the relationship between mean pollen load per population and mean population fruit and seed set to address whether reproduction may be pollen-limited. A linear relationship between mean pollen load and mean fruit set was not significant across all populations ($F_1 = 3.30$, $P = 0.129$). Instead, mean fruit set appeared to saturate as pollen load reached about 450 grains or higher (Fig. 3). Across those populations with mean pollen loads less than this amount, a linear relationship was significant and explained 91% of the variation in mean fruit set ($F_1 = 30.81$, $P = 0.012$). There was a significant, positive relationship between mean pollen load and mean seed set ($F_1 = 10.53$, $P = 0.018$) (Fig. 3). This linear relationship was strong and explained approximately 64% of the variation in seed set across populations.

Discussion

POPULATION-LEVEL EFFECTS

Population size explained significant variation in mean fruit set per population in *S. angularis*. Small populations exhibited reduced fruit set, but the effect of population size across the entire range of population sizes was non-linear. This result is distinct from the positive, linear relationship between reproduction and population size seen in other studies (e.g. Ågren 1996; Luijten *et al.* 2000; Hackney & McGraw 2001; Wagenius 2006). In those studies, plants benefited reproductively from increases in population size on a continuous basis. Instead, we found that the beneficial effects of increased population size on fruit set in *S. angularis* begin to cease beyond some point and may even begin to decline with further increases in population size. Declines in fruit set in larger populations might arise if individuals faced increased competition or if large populations had higher herbivore or pathogen loads (e.g. Bach 1984; Colling & Matthies 2004; Östergård & Ehrlén 2005). Despite these possibilities, there is greater support for the saturation model. This support comes from the superior fit of this model describing the relationship between population size and mean fruit set compared to a quadratic model. In addition, anecdotal support comes from an estimate of fruit set in an extremely large *S. angularis* population comprising over 7000 individuals, which was not included in this study. Fruit set in this population was approximately 64% (R. Spigler, unpubl. data) and suggests that even if fruit set declines with increasing population size, the rate of decline is likely to be negligible between the largest population included in this study and a population over 20 times larger.

An important result from the fruit-set data is that we are able to define the size at which populations are likely to face significant reductions in fruit set. The saturation model reveals a critical threshold population size of about 15 individuals, below which fruit set drops precipitously. Fruit set in these small populations can reach as low as 45% and, on average, is only 60% compared to an average of 81% in larger populations.

Reduced fruit set in small populations could be the result of pollen limitation. Previous research has demonstrated pollen-limited fruit and seed set in natural *S. angularis* populations (Dudash 1993). In our study, fruit set increased sharply with pollen load up to approximately 450 grains, consistent with pollen-limited reproduction up to that point. Beyond this amount, the data suggest that 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. However, maximum fruit set in the study populations did not reach this high. Instead, the apparent saturation may 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 appears 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. However, rather than changing linearly with population size, it is possible that pollen load drops abruptly below a certain size, as was seen for fruit set. This effect was demonstrated most strikingly in a study on the annual *Clarkia concinna* (Groom 1998). Individuals in the smallest *S. angularis* study population included in the pollen-load study did have less pollen per stigma, on average, than individuals in the other seven populations combined; however, this amount was only significantly less than mean pollen loads in populations JRM and CFR (data not shown). Given that only the smallest *S. angularis* populations have reduced fruit set, stigmas from additional populations with less than 15 individuals would improve our ability to evaluate this hypothesis.

Alternative mechanisms to explain the reductions in fruit set in small *S. angularis* populations exist, but are less likely. For example, increased autonomous self-fertilization in self-compatible *S. angularis* in response to reduced pollinator visitation could have caused the observed reduction in fruit set if inbred fruits were aborted selectively. However, neither fruit nor seed set are affected by inbreeding depression in this species (Dudash 1990; Spigler 2007). Alternatively, reduced fruit set in small populations could occur if those populations were 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). Yet 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 ($r_{\text{height},18} = -0.05$, $P = 0.85$; $r_{\text{buds},18} = 0.04$, $P = 0.87$) (R. Spigler, unpubl. data). Experimental manipulations of population size are needed to elucidate the mechanism responsible for reduced fruit set in small populations of *S. angularis*.

The effect of population size on fruit set did not persist for RS. This may be because population size did not predict seed set at the population level. Instead, variation in seed set was better explained on local spatial scales. In general, RS of populations with less than 15 individuals appeared to be variable, whereas the remaining populations followed a linear relationship with population size. Mean RS in small populations was as low as approximately 970 seeds, which is over 8000 less than the mean across all populations. However, the RS of population BCE (with only one individual) was comparable to that of population LI1 (with 57 individuals) and population LI2 (with 76 individuals). Thus, population size is not useful for predicting mean population RS.

However, population density was able to explain significant variation in mean population RS, despite perhaps being too weak to affect either mean fruit or seed set independently and despite representing a crude estimate of plant spacing. Yet in contrast to our initial predictions, the relationship between density and mean RS was negative: low-density populations enjoyed the highest success. Similarly, Mustajärvi *et al.* (2001) found lowered capsule production in dense populations of

Lychnis viscaria relative to sparse populations. These patterns might arise if competition for resources increases with density, reducing per-capita resource availability in high-density populations. Indeed, the negative effects of intra-specific interactions via competition in large populations are well documented within the plant literature (Harper 1977; Weiner 1982; Pacala & Silander 1985; Silander & Pacala 1985). Alternatively, reduced RS in high-density *S. angularis* populations could be the result of competition for pollinators (e.g. Rathcke 1983), but this explanation is not supported by the pollen-load data. We caution that the very smallest populations, consisting of only one or two plants, were not included in the density analyses because the population-level density measures used in this study would have been inappropriate for such small population sizes.

LOCAL-LEVEL EFFECTS

Per-capita fruit set increased linearly with local neighbourhood size at both the 1-m and 4-m spatial scales. These effects may indicate that a greater number of neighbours facilitates pollination or merely that larger neighbourhoods represent resource-rich patches. These two mechanisms are not mutually exclusive, and, in fact, greater resource availability could beget greater pollen production (e.g. Lau & Stephenson 1993). Although not significant, increases in the number of additional neighbours at the 4-m spatial scale tended to increase individual pollen load and may be partially responsible for greater fruit set at this scale as well. However, an interaction between plant height and 4-m LNS suggests that the effect of local neighbours is not straightforward and instead is contingent upon focal plant height, and thus perhaps the degree of competition among neighbours.

While a greater number of neighbours served to increase fruit set, the opposite trend occurred for seed set. At the smallest spatial scale, increases in local neighbourhood size within 1 m of focal plants actually decreased seed set. This linear decrease is highly suggestive of intra-specific competition for resources. Interactions at this scale may have changed from positive, as was seen for fruit set, to negative if competition for resources increased later in the season, after fruits had begun to develop but during seed maturation. However, the negative effect of increased LNS was not evident beyond the 1-m scale. Such fine spatial resolution of the underlying interactions may explain, in part, why a relationship between mean seed set and plant abundance was not captured at the population-level spatial scale.

The effect of LNS on subsequent RS was scale-dependent, representing the combined effects of LNS on fruit and seed set. At the smallest spatial scale, greater LNS decreased RS. The net negative effect of 1-m LNS suggests that the negative effects of competition occurring during seed set were strong enough to outweigh the facilitative interactions for fruit set at that scale. Yet beyond 1 m, increasing neighbourhood size at the 4-m scale increased RS significantly. As was suggested for fruit set, this result may be caused by increased pollinator visitation in large patches, such as was found for the perennial

wildflower *Lesquerella fendleri* (Roll *et al.* 1997). However, a significant interaction between plant height and 4-m LNS again indicates that the effect of neighbourhood size at this scale is context-dependent. The opposing effects at the 1-m and 4-m spatial scales further 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.

Conclusions

The results of this study revealed a non-linear relationship between reproduction and population size. Fruit set in *S. angularis* populations is reduced significantly in populations with less than approximately 15 individuals, but remains relatively constant in larger populations. Furthermore, the sharp decline in the non-linear relationship provides a practical definition for 'small' with respect to population size for *S. angularis*. In addition, we found that the consequences of variation in plant abundance for reproduction in *S. angularis* were scale-dependent. Wagenius (2006) also examined the effect of plant abundance over multiple local and population spatial scales on reproduction in purple coneflower (*Echinacea angustifolia*), a long-lived perennial affected by fragmentation of North American tall-grass prairies, but found that reproduction increased with population size at all scales examined. In contrast, overall population size, density, 1-m LNS and 4-m LNS 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. Experimental investigation isolating the role of population size and local neighbourhood on reproduction would help elucidate which interactions are likely to drive the relationships seen in nature.

This study adds to the body of evidence documenting the effect of plant abundance on reproduction. We found that small populations of *S. angularis* experience decreased fruit set. This pattern supports the emerging principle that reproduction is reduced in small plant populations and is consistent with other studies on much less common species, such as the rare plants *Gentianella germanica* (Fischer & Matthies 1998) and *Primula vulgaris* (Brys *et al.* 2004) and the endangered plant *Rutidosia leptorrhynchoides* (Morgan 1999). However, this pattern was not pervasive for subsequent reproductive success, presumably because of opposing facilitative and competitive interactions occurring on local spatial scales. Instead, reproductive success in small *S. angularis* populations was unpredictable and may therefore be less stable than reproductive success in larger populations. Furthermore, population size and density affected reproduction differently, underscoring that these measures can influence reproduction via different mechanisms. Total reproductive success declined with increasing density, suggesting that while small populations may suffer decreases in fruit set, plant density and competitive interactions may be more important in determining subsequent reproductive success at the population level.

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