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THE EFFECT OF POPULATION SIZE ON STIGMA POLLEN LOAD, FRUIT SET, AND SEED SET IN *ALLIUM STELLATUM* KER. (LILIACEAE)

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In *Allium stellatum*, a xenogamous self-compatible prairie species, we examined stigma pollen load, percentage of fruit set, and percentage of seed set in large and small populations over 2 yr. We tested for significant effects of population size on these characteristics, for relationships between stigma pollen loads and fruit and seed set, and for significant variation between years in reproductive characteristics. In 1994 stigma pollen loads were inversely related to population size and were significantly lower than in 1993. The lower stigma pollen loads in 1994 were associated with adverse weather conditions (high rainfall), which are known to affect pollinator activity and may have led to competition among plants for pollinators in large populations. However, population size was not correlated with percentage of fruit set or percentage of seed set in either year. The lack of fruit and seed set differences between large and small populations, particularly in 1994, likely resulted from the small amount of pollen needed to trigger fruit and seed production. Our results indicate that for some self-compatible species that similarly require small pollen loads, the effects of population size on reproduction are minimal, even if differences in pollen-movement exist between large and small populations.

Keywords: population size, reproduction, prairies, *Allium stellatum*.

Introduction

Two of the main consequences of habitat loss for plant populations are isolation from other conspecific populations and reduction in population size (Wilcove et al. 1986; Saunders et al. 1991; Lamont et al. 1993; Hall et al. 1996; Risser 1996). The resulting small or isolated populations often have decreased fruit set, seed set, or seed germination relative to large populations (Menges 1991; Byers and Meagher 1992; Hendrix 1994; Heschel and Paige 1995; Ågren 1996). Although reductions in the reproductive success of outbreeding plant species may be attributable to inbreeding depression (Franklin 1980; Heschel and Paige 1995) or genetic erosion (Oubourg et al. 1991; van Treuren et al. 1991), changes in plant-pollinator interactions may be equally important (Rathcke and Jules 1993). Several studies have shown that the foraging behavior of pollinators and the number of pollinators are negatively affected by habitat loss (Jennersten 1988; Aizen and Feinsinger 1994; Rathcke and Jules 1993) and increased distance between studied areas (Powell and Powell 1987; Spears 1987; Rathcke and Jules 1993), thus resulting in lower plant reproductive success.

Although both pollinators and the reproductive success of plants in small populations may be negatively affected by habitat fragmentation (Jennersten 1988; Kwak 1988; Petanidou et al. 1995), relatively few studies have directly shown that decreased pollinator activity and reproduction are connected through the process of pollen movement, particularly the number of pollen grains moved (Feinsinger et al. 1991; Kunin 1993; Byers 1995). Low fruit set may be caused by a variety of factors, such as resource availability, unrelated to pollen movement, and even low pollinator abundance may be sufficient to move adequate amounts of pollen to complete the fertilization of available ovules, especially when the number of ovules per flower is small.

We examined the effect of population size on stigma pollen load, fruit set, and seed set in *Allium stellatum* (Liliaceae). Specifically, we addressed the following three questions. First, is there significant variation between populations of different sizes in stigma pollen load, fruit set, and seed set? Second, is fruit or seed set related to pollen stigma loads? Finally, is there significant variation between years in these characteristics?

Material and Methods

Study Species

Allium stellatum Ker. (Liliaceae) is a prairie perennial found on barren and rocky hills, ranging from western Ontario to Illinois, west to Saskatchewan, Wyoming, and south to Oklahoma (Churchill 1986). In Iowa, it is restricted to prairie fragments in the northwest portion of the state (Eilers and Roosa 1994). Plants are 2–6 dm tall with a membranaceous coated bulb. The flowering period in Iowa is late July to September. The protandrous flowers of *A. stellatum* are pink, with six anthers and a superior ovary containing six ovules. The flowers are arranged in 25–50 umbels and open sequentially, beginning with the central flowers. The pollinators of *A. stellatum* are generalists such as solitary bees and syrphid flies (B.

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Molano-Flores, personal observations). The fruit is a three-lobed capsule. Preliminary studies of the mating system indicate that this species is xenogamous and self-compatible with a pollen : ovule ratio of 900 (Cruden 1977; B. Molano-Flores, unpublished data).

Study Sites and Data Collection

We conducted this study in 1993 and 1994 in Dickinson and Emmet Counties, Iowa. To assess the effect of population size of *A. stellatum* on mean stigma pollen load, mean percentage of fruit set, and mean percentage of seed set, in 1993, we selected three large populations (>1000 individuals) on three state preserves and 13 small populations (3–60 individuals) along unimproved roads in Dickinson County (table 1). The three preserves sampled were Cayler Prairie and Freda Haffner Preserve in Dickinson County and Anderson Prairie in Emmet County. The roadside populations were within a matrix of prairie species, indicating a small prairie remnant. In 1994, we resampled the three large populations and three of the small populations. The remaining 10 small populations were mowed or sprayed and could not be resampled.

In each population, we determined the number of flowering individuals at the peak of the flowering season. In all populations, the area occupied was mapped and measured each year. We defined effective population size for reproduction as the absolute number of flowering individuals, and we measured it by direct counting in small populations. In large populations, we estimated population size from area measurements and the number of flowering individuals in 100 1-m^2 quadrats regularly placed along parallel transects located throughout the population.

To determine mean stigma pollen load per population, we collected one flower with a receptive stigma from each of 20 plants in large populations and from 10 to 20 plants (depending on population size) in small populations. Flower collections for all populations were done the same day during the peak of the flowering season (i.e., September 12, 1993, and September 28, 1994). These days were sunny with at least 2 d of no precipitation prior to the date of flower collection (U.S. Environmental Data Service 1993–1994). Flowers were stored in 1 : 3 solution of acetic acid : 95% ethanol. We removed the pistils from the flowers and prepared them for fluorescence microscopy by soaking them in 1 N NaOH for 12 h to soften the tissue and staining them with 0.1% aniline blue in 0.1 M K_3PO_4 for 12 h (Martin 1959). We mounted the pistils on slides in glycerol and scored them for total number of pollen grains per stigma.

To estimate percentage of fruit set, we collected 20 infructescences (i.e., single umbels) from different plants in large populations and 2–20 infructescences in small populations. We calculated percentage of fruit set per population as the mean percentage of flowers per umbel setting one or more seeds. We estimated percentage of seed set per population by selecting 10 fruits from each infructescence and determining the mean percentage of ovules per infructescence that developed into mature seeds. Mature, fully developed seeds are plump and dark in color; partially developed seeds are concave and lighter in color (B. Molano-Flores, personal observations).

Table 1

Location, Remnant Type, and Population Size (No. of Flowering Individuals) of *Allium stellatum* in 1993 and 1994

Population address	Remnant type	1993	1994
Freda Haffner Preserve	State preserve	1044	1061
Cayler Prairie	State preserve	1100	1035
Anderson Prairie	State preserve	1125	1014
170 St. No. 1	Roadside	10	3
215 Ave. No. 1	Roadside	11	10
215 Ave. No. 2	Roadside	26	17
180 St. No. 1	Roadside	19	...
130 St.	Roadside	5	...
120 St. No. 1	Roadside	56	...
120 St. No. 2	Roadside	29	...
120 St. No. 3	Roadside	8	...
210 Ave.	Roadside	3	...
140 St.	Roadside	15	...
170 St. No. 2	Roadside	9	...
180 St. No. 2	Roadside	4	...
120 St. No. 4	Roadside	26	...

Statistical Analysis

For the six populations sampled in both years, we used repeated-measures ANOVA (RM-ANOVA) to examine effects of population size (>1000 vs. <100) and year on stigma pollen load, percentage of fruit set, and percentage of seed set; all dependent variables were population-level means. We report univariate tests of within-subject effects; results based on multivariate tests do not differ. Because we also had data for 10 populations sampled in only 1 yr, we examined population size effects further using regression analyses (log-transformed population size vs. mean stigma pollen load, percentage of fruit set, and percentage of seed set) within each year. After detecting a relationship between population size and mean stigma pollen load in 1994, we used *G*-tests to examine the association between population size and the frequency of plants receiving no pollen. Finally, we tested for relationships between stigma pollen loads and fruit and seed sets in each year using regression analyses across populations.

Because the population size data were severely nonnormal and because any variables depending on population size might therefore be nonnormal, we evaluated the significance of RM-ANOVA and regression test statistics by randomization (Manly 1991). We compared actual test statistics to a distribution calculated from 1000 randomized data sets. For regressions, randomizations and analyses were conducted using a computer program written in QuickBASIC (Microsoft 1988). For RM-ANOVAs, randomizations were done using a second QuickBASIC program and the analyses were conducted using SAS version 6.03 (SAS Institute 1988).

Statistical power was rather low for tests using only the six resampled populations and for regressions among populations in 1994. Power was good for regressions among populations in 1993 (power > 0.9 at $\alpha = 0.05$ for true $r^2 = 0.49$).

Table 2

Mean Stigma Pollen Load, Percentage Fruit Set and Percentage Seed Set in *Allium stellatum* Populations in 1993 and 1994 (±SD)

Population address	Population size	Stigma pollen load		Fruit set (%)		Seed set (%)	
		1993	1994	1993	1994	1993	1994
Freda Haffner Preserve	Large	8.20 (11)	3.77 (7)	65.6 (14)	58.5 (12)	44.5 (13)	50.5 (12)
Cayler Prairie	Large	10.91 (17)	1.93 (4)	65.9 (16)	48.4 (22)	39.2 (10)	39.8 (14)
Anderson Prairie	Large	11.84 (15)	1.90 (4)	54.8 (17)	39.4 (17)	33.5 (10)	29.4 (16)
170 St. No. 1	Small	2.50 (3)	8.33 (10)	61.8 (9)	43.5 (7)	39.4 (11)	41.7 (9)
215 Ave. No. 1	Small	17.17 (14)	4.00 (3)	58.3 (8)	51.0 (14)	38.3 (9)	40.3 (5)
215 Ave. No. 2	Small	25.76 (24)	6.50 (8)	66.4 (14)	40.5 (19)	46.7 (13)	45.2 (11)
180 St. No. 1	Small	4.18 (12)	...	72.5 (15)	...	45.8 (6)	...
130 St.	Small	7.67 (11)	...	50.0 (15)	...	35.3 (7)	...
120 St. No. 1	Small	...	...	67.4 (12)	...	44.3 (14)	...
120 St. No. 2	Small	3.67 (5)	...	45.6 (24)	...	42.5 (20)	...
120 St. No. 3	Small	...	...	53.0 (13)	...	36.5 (7)	...
210 Ave.	Small	7.33 (10)	...	15.8 (0)	...	33.0 (0)	...
140 St.	Small	11.75 (10)	...	47.3 (27)	...	25.8 (10)	...
170 St. No. 2	Small	7.13 (7)	...	56.0 (12)	...	46.2 (13)	...
180 St. No. 2	Small	12.38 (4)	...	65.8 (9)	...	23.0 (11)	...
120 St. No. 4	Small	21.0 (20)	...	71.2 (9)	...	36.1 (4)	...

Results

Among the six resampled populations, mean stigma pollen load showed a trend toward lower loads in 1994 than 1993 ($F_{1,4} = 4.6$, $P = 0.098$). On average, stigma pollen loads in 1994 ($\overline{X} \pm \text{SD} = 4.4$ grains $\pm$ 2.6) were less than one-half those in 1993 ($\overline{X} \pm \text{SD} = 10.8$ grains $\pm$ 6.7) (table 2). There was no detectable effect of population size and no size × year interaction ($F_{1,4} < 1.92$, $P > 0.40$). However, regressions within each year using the complete data set revealed a significant negative relationship between population size and stigma pollen loads in 1994 (fig. 1) but no relationship in 1993 ($r^2 = 0.004$, $P = 0.84$). The significant relationship in 1994 was driven largely by the high frequency of flowers that received no pollen in the three large populations ($G = 18.2$, 1 df, $P < 0.001$; fig. 2*b*). Among plants receiving at least some pollen, the frequency distribution of stigma pollen load was similar in large and small populations ($G = 1.4$, 1 df, $P > 0.5$). In 1993 there were no differences between large and small populations in the frequency distribution of stigma pollen load ($G = 0.37$, 1 df, $P > 0.5$; fig. 2*a*).

Fruit set among the six resampled populations, measured as the percentage of flowers per umbel setting one or more seeds, was significantly lower in 1994 ($\overline{X} \pm \text{SD} = 46.9\% \pm 4.7\%$) than in 1993 ($\overline{X} \pm \text{SD} = 62.1\% \pm 7.2\%$) ($F_{1,4} = 23.7$, $P = 0.013$; table 2). There were no population size or size × year effects ($F_{1,4} < 0.38$, $P > 0.57$). Even with the larger sample of populations in 1993, there was no population size effect ($r^2 = 0.12$, $P = 0.19$).

Seed set (table 2), measured as the percentage of ovules per fruit developing into mature seeds, was not affected by population size or year, either among the six resampled populations (all $F_{1,4} < 0.32$, $P > 0.57$) or among the full set of populations in 1993 ($r^2 = 0.07$, $P = 0.33$). Seed set ranged from 23% to 51%. Neither fruit set nor seed set depended on stigma pollen load in either year ($-0.22 < r^2 < 0.18$, both $P > 0.29$).

Discussion

Several studies have demonstrated that decreased reproduction by plants in small populations is correlated with decreased pollen movement (Feinsinger et al. 1991; Kunin 1993; Byers 1995), supporting numerous suggestions that changes in plant-pollinator interactions are an important aspect of the fragmentation process affecting plant population viability (Rathcke and Jules 1993; Aizen and Feinsinger 1994; Fritz and Nilsson 1994; Hendrix 1994; Heschel and Paige 1995). In

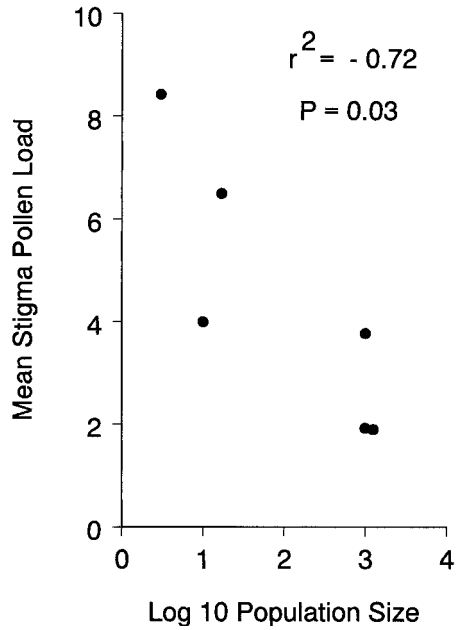

Fig. 1 Relationship between population size and mean stigma pollen load in *Allium stellatum* populations in 1994.

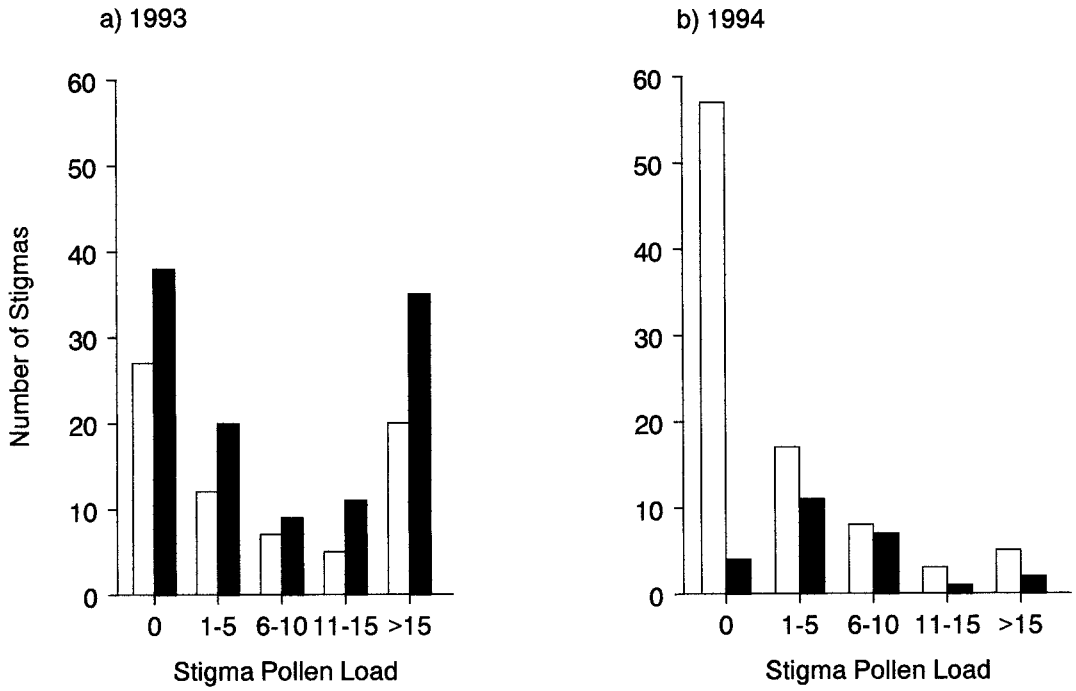

Fig. 2 Frequency distribution of stigma pollen load in large (open bars) (>1000 flowering ramets) versus small (filled bars) (<100 flowering ramets) populations of *Allium stellatum* in (*a*) 1993 and (*b*) 1994.

contrast to these studies, we found no evidence that pollen delivery differences within years between small and large populations are correlated with either percentage of fruit set or percentage of seed set in *Allium stellatum*. However, we did find differences between the 2 yr of this study in pollen movement across all population sizes in which relatively low pollen movement in 1994 was associated with relatively low percentage of fruit set.

Differences between years in stigma pollen loads in *A. stellatum* appear to be linked to weather conditions affecting pollinator activity. In particular, differences between years in September rainfall (most flowering occurs in September for *Allium* at our sites) may have driven differences in pollination. In 1993, when stigma pollen loads were relatively high, rainfall at Esterville, Iowa (the weather station nearest Anderson Prairie), was 53% of normal (4.32 cm vs. 8.46 cm) (U.S. Environmental Data Service 1993–1994), allowing for greater pollinator activity. Milford, Iowa (the weather station nearest to Cayler Prairie and the other sites), had a similar pattern of rainfall in 1993. In contrast, rainfall at Esterville in 1994 was 132% of normal (11.15 cm), and although rainfall at Milford in 1994 was 85% of normal (7.75 cm), 41% fell during the 2-wk period of greatest flowering in late September. We hypothesize that the increased rainfall during September 1994 depressed pollen arrival to stigmas of *A. stellatum* by decreasing overall levels of pollinator activity or by altering the composition of the pollinator guild such that the most effective pollinators of *A. stellatum* were less abundant than in 1993. It is also possible that the greater than normal rainfall in May and June 1993 (218% and 278% of normal, respectively) (U.S. Environmental Data Service 1993–1994) may have negatively affected long-term population dynamics of pollinators, thereby decreasing their abundance in 1994 (R. Cruden, personal communication). A similar negative effect of rainfall on pollinator activity has been found with *Phlox pilosa* (Khyl 1997) and *Anemone canadensis* (Molano-Flores 1997), indicating that this is not an isolated phenomenon.

Differences in rainfall between years resulting in variation in pollinator activity or abundance can also account for differences between years in the relationship between population size and pollen arrival. In September 1994, when rainfall was relatively high, we hypothesize that decreased pollinator activity led to competition for pollinators between plants in large populations, resulting in a negative relationship between population size and mean stigma pollen load. In 1993, when stigma pollen loads were more than twice of those in 1994 (table 2), we hypothesize that no such competition for pollinators occurred, accounting for the lack of a relationship between population size and stigma pollen load.

The lack of differences in fruit set and seed set between large and small populations of *A. stellatum* may be related to two nonexclusive reasons. First, while self-pollination after flower collection might have increased stigma pollen loads, we find this unlikely since *A. stellatum* is protandrous. Second, even though large populations may receive fewer pollen grains per stigma than small populations, the number is sufficient to produce substantial fruit and seed set. The results of our study strongly indicate that any functional effects of reductions in plant population size related to habitat fragmentation and hypothesized to be related to changes in plant-pollinator interactions should be documented with analyses of stigma pollen load as well as seed and fruit set over more than a year.

Other factors, such as seed predators, may play an important role in the persistence or extinction of *A. stellatum* populations. In both large and small populations of *A. stellatum*, the larvae of an unidentified moth heavily damaged many fruits, indicating possible seed-recruitment problems within the populations. Stochastic events may also affect these populations, especially small ones. For example, in 1994, only three of the 10 small populations that were studied in 1993 were resampled because of mowing or roadside spraying of herbicides. Such factors may reduce the long-term viability of these small populations by reducing contributions to any potential seed bank. When such stochastic events occur in conjunction with years of low fruit set, they may cause more local population extinctions of *A. stellatum* than changes in biotic interactions alone would indicate.

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