

Relationships between the floral neighborhood and individual pollen limitation in two self-incompatible herbs

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Abstract Local flower density can affect pollen limitation and plant reproductive success through changes in pollinator visitation and availability of compatible pollen. Many studies have investigated the relationship between conspecific density and pollen limitation among populations, but less is known about within-population relationships and the effect of heterospecific flower density. In addition, few studies have explicitly assessed how the spatial scales at which flowers are monitored affect relationships. We investigated the effect of floral neighborhood on pollen limitation at four spatial scales in the self-incompatible herbs *Armeria maritima* spp. *maritima* and *Ranunculus acris* spp. *acris*. Moreover, we measured pollen deposition in *Armeria* and pollinator visits to *Ranunculus*. There was substantial variation in pollen limitation among *Armeria* individuals, and 25% of this variation was explained by the density of compatible and heterospecific flowers within a 3 m circle. Deposition of compatible pollen was affected by the density of compatible and incompatible inflorescences within a 0.5 m circle, and deposition of heterospecific pollen was affected by the density of heterospecific flowers within a 2 m circle. In *Ranunculus*, the number of pollinator visits was affected by both conspecific and heterospecific flower densities. This did not, however, result in effects of the

floral neighborhood on pollen limitation, probably due to an absence of pollen limitation at the population level. Our study shows that considerable variation in pollen limitation may occur among individuals of a population, and that this variation is partly explained by floral neighborhood density. Such individual-based measures provide an important link between pollen limitation theory, which predicts ecological and evolutionary causes and consequences for individual plants, and studies of the effects of landscape fragmentation on plant species persistence. Our study also highlights the importance of considering multiple spatial scales to understand the spatial extent of pollination processes within a population.

Keywords Competition · Facilitation · Flower density · Pollination

Introduction

Local abundance and density of plant species decrease when human activity causes degradation and fragmentation of habitats (Lienert 2004). This, in combination with the ongoing decline in pollinator populations (Kearns et al. 1998), makes it important to understand how changes in flower density influence plant–pollinator interactions and the resulting reproductive output of plants (e.g., Kunin 1997a; Knight 2003; Kirchner et al. 2005). Many studies have investigated the effects of population size and density on mean population pollination success (reviewed in Kunin 1997b), but comparatively few have considered individual-based measures where the local floral neighborhood is related to the reproduction success of single individuals (e.g., Roll et al. 1997; Wagenius 2006; Spigler and Chang 2008). Both perspectives are of

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importance for understanding the causes of plant reproduction failure (Wagenius 2006).

Individual-based measures require the delimitation of the effective floral neighborhood, which is unknown in most cases. The effective floral neighborhood may depend on search behavior, foraging range and diet breadth of the pollinators of the plant species, and data on this are often absent (Ghazoul 2005). A way to handle this problem is to assess flower density at different distances from the target individual. Studies which have adopted this approach show that the spatial scale at which the floral neighborhood is estimated can have large effects on the relationships found (e.g., Roll et al. 1997; Spigler and Chang 2008; Wagenius 2006).

Conspecific flower density affects the seed production of animal-pollinated plants through attraction/competition for pollinators, competition for light, water and nutrients, and by affecting the number of potential mates. A positive relationship between population density and reproduction or survival, the Allee effect (Allee et al. 1949), has been demonstrated in many studies (e.g., Kunin 1992; Wolf and Harrison 2001; Elam et al. 2007), and can be explained by a positive effect of flower density on pollen availability and pollinator visitation. An increase in the density of conspecific flowers will always increase the availability of compatible pollen, but could affect pollination either positively (Ehlers et al. 2002) or negatively (Mustajärvi et al. 2001) depending on the net effect on visitation rates. A model of density-dependent visitation was suggested by Rathcke (1983), who predicted that an increase in flower density would have a positive effect on per-flower visitation rate at low densities and a negative effect at high densities, when flower number increases faster than the number of pollinator visits. Such a hump-shaped response was found between conspecific flower density and fruit set in a deceptive orchid, where seed set peaked at intermediate flower densities and was low at lower and higher densities (Sabat and Ackerman 1996).

Rathcke's model also applies to interactions for pollinators among plant species, where pollinator sharing can cause density-dependent effects similar to those within a species (Rathcke 1983). However, effects of heterospecific flower density on plant reproduction are much less studied than effects of conspecific density. Mostly competitive (Chittka and Schürkens 2001; Brown et al. 2002; Bell et al. 2005; Totland et al. 2006) but also facilitative (Schemske 1981; Moeller 2004) effects have been found among pollen- and/or nectar-rewarding plant species. A special case of facilitation is the "magnet-species effect," where nonrewarding plant species pollinated by deceit receive more visits when they grow close to attractive plant species than when they grow alone (Lavery 1992; Johnson et al. 2003; Juillet et al. 2007). A high density of heterospecific flowers

can affect pollination negatively through improper pollen transfer, which occurs when pollinators switch between species (Galen and Gregory 1989; Caruso and Alfaro 2000; Jakobsson et al. 2007). Heterospecific pollen can cause decreased seed production through chemical interference (Sukhada and Jayachandra 1980; Murphy 2000) or stigma surface blocking (Rathcke 1983; Galen and Gregory 1989).

Pollen limitation occurs when plants produce fewer seeds or seeds of lower quality than when they receive adequate pollination, and thus comprises both a quantity and quality limitation on reproductive success (e.g., Wilcock and Neiland 2002; Aizen and Harder 2007). Pollen limitation is usually estimated by comparing the seed production of pollen-supplemented flowers (assumed to have received 100% pollination) with the seed production of naturally pollinated flowers (Larson and Barrett 2000), and refers to a decrease in plant reproductive success that is distinguished from effects of resource limitation (e.g., Knight et al. 2006). The relationship between flower density and pollen limitation has most commonly been studied at the among-population level and has concerned only conspecific flower density. Most of these studies have found a negative relationship between conspecific density and the magnitude of pollen limitation (Steven et al. 1999; Karrenberg and Jensen 2000; Moody-Weis and Heywood 2001; Knight 2003; Wailes and Ågren 2004; Kolb 2005), although positive relationships (Larson and Barrett 1999; Baker et al. 2000) and nonsignificant relationships (Lázaro and Traveset 2006) also occur. Few studies have investigated the relationship between local flower density and pollen limitation among individuals within the same population (but see de Jong et al. 2005; Wagenius 2006; Zorn-Arnold and Howe 2007), and we know of no study that also has assessed the effects of heterospecific flower density on individual-level pollen limitation.

We examined the relationship between natural variation in conspecific and heterospecific flower density and individual pollen limitation in two self-incompatible herbs: *Armeria maritima* spp. *maritima* (*Armeria* hereafter) and *Ranunculus acris* spp. *acris* (*Ranunculus* hereafter). We will refer to this conspecific and heterospecific flower density as the "floral neighborhood" throughout the paper. Thus we use this expression in a more general sense than it is usually employed in pollination biology, where it denotes a genetic neighborhood that depends on pollen dispersal distance (Wright 1946). We also investigated how these relationships were affected by the spatial scale at which the floral neighborhood was measured. In addition, to reveal the underlying processes through which neighbors affect pollen limitation, we investigated the effect of conspecific and heterospecific flower density on pollen deposition (in *Armeria*) and pollinator visitation (in *Ranunculus*). Specifically, we asked: (i) are populations of these two

species pollen limited? (ii) Does pollen limitation vary among individuals of the same population? (iii) Does the floral neighborhood explain variation in pollen limitation among plants? (iv) Does the spatial scale at which the floral neighborhood is monitored affect these relationships? (v) Does the floral neighborhood explain variation in pollen deposition among plants? (vi) Is pollinator visitation affected by conspecific and heterospecific flower density?

Methods

Study species and field sites

Armeria maritima spp. *maritima* (Plumbaginaceae) has a diallelic sporophytic self-incompatible system (i.e., two separate morph types and no mating within each morph type). One morph is heterozygous with a “cob-like” stigma and coarsely reticulate pollen; the other morph is homozygous with a papillate stigma and finely reticulate pollen (Richards 1997). The species is perennial, bearing leaves in a basal rosette, and the pink open flowers are clustered in dense inflorescences. Its main flowering period is from May to June and each flower develops a one-seeded indehiscent fruit. The study of *Armeria* was performed in the Stångehuvud nature reserve, a small peninsula on the west coast of Sweden (N 58.2709 E 011.4208), between 17 May and 26 June in 2007.

Ranunculus acris spp. *acris* (Ranunculaceae) has a gametophytic self-incompatible system (Richards 1997). The species is a perennial geophytic herb and bears large yellow bowl-shaped flowers on erect stalks. It flowers from May to June and each flower has several ovaries (ranging between 12 and 33 in our population), which may develop into a one-seeded indehiscent fruit (achene). The study of *Ranunculus* was performed in meadows in Torgestad, on the west coast of Sweden (N 58.3409 E 011.5410), between 31 May and 14 July in 2007.

Both species are reported as being self-incompatible, but this may differ among geographical regions and populations. Therefore, we investigated the frequency of selfing in the populations by comparing the seed set of flowers with pollinator visits prevented to the seed set of flowers subjected to natural pollination. Ten individuals of *Armeria* and 25 individuals of *Ranunculus* were chosen haphazardly, and we used empty tea-bags (Beste Tassen Filter, die Tee Familie) to prevent pollinator visitation. We bagged one inflorescence on each *Armeria* individual ($n = 10$), and one flower on each *Ranunculus* individual ($n = 25$), before the flowers had opened. We marked control inflorescences/flowers on the same individuals, but on separate branches. The inflorescences/flowers were haphazardly selected for the different treatments.

Pollen limitation

We examined pollen limitation in an experiment where the seed set of pollen-supplemented flowers (i.e., hand-pollinated flowers that were also open for natural pollination) was compared to the seed set of naturally pollinated flowers on the same plant. We performed hand pollinations on 50 *Armeria* individuals and 75 *Ranunculus* individuals. In each *Armeria* individual, pollen was supplemented on three inflorescences and three inflorescences were marked as naturally pollinated controls. All flowers in an inflorescence were subjected to the pollination treatment. Individuals were randomly selected by choosing the first individual we encountered after moving 9 m from the last individual (to keep the 3 m circles independent of each other). Inflorescences were haphazardly assigned to the pollination treatment. We identified the morph identities of the pollen recipient and donor individuals in the field with a pocket microscope (Lumagney) with 60–100× magnification. Pollen donors were situated >15 m from their recipients, and we transferred the pollen by gently striking the stigmas with open, pollen-laden anthers. Flowers received pollen from only one pollen donor each day, but most flowers received pollen from two or three donors, and inflorescences received pollen from at least eight donors, since pollen supplementation was performed daily or every second day (depending on the weather conditions) until all of the flowers in an inflorescence had wilted. The time elapsed from the first to the last supplemental pollination of *Armeria* was 24 days. After the last flower wilted we enclosed all inflorescences in tea-bags to prevent the seeds from dispersing.

We selected *Ranunculus* individuals by the same method as for *Armeria* (see above), and on each individual we haphazardly selected one flower branch for natural pollination and another for pollen supplementation. We pollinated all flowers on a branch, which ranged from four to seven in number. Each flower was pollinated 2–4 times depending on the weather conditions (during hot and warm weather the flowers wilted faster). We used the same method for pollen collection and transfer as described for *Armeria*. Flowers on the same branch opened more or less sequentially, and the duration from the first to the last supplemental pollination in *Ranunculus* was 19 days. Accordingly, seedheads ripened sequentially and we therefore collected seedheads on five different occasions. The seed collection time reflects the flowering time; i.e., seedheads collected first originated from the first flowers to open. We collected each seedhead separately, and seedheads that had lost several seeds before collection were omitted from the analyses.

The numbers of filled and unfilled seeds were counted in the lab. For *Ranunculus*, we also identified half-filled seeds that were fertilized but aborted. Unfortunately, aborted

seeds could not be identified in *Armeria*. A few seeds of both species were damaged by seed predators, but it was possible to distinguish these from unfilled seeds. We calculated the seed set of an inflorescence or flower as $S = S_f / O_{tot}$, where S_f is the number of filled seeds and O_{tot} is the total number of ovules. In *Ranunculus*, we also calculated the fertilization rate as $F = O_f / O_{tot}$, where O_f is the number of fertilized ovules (sum of aborted and filled) and O_{tot} is the total number of ovules. There was a strong correlation between fertilization rate and seed set in *Ranunculus* ($r = 0.95$, $P < 0.001$). We consider fertilization rate to be the most relevant estimate of pollination success (since abortion of seeds could be due to resource deficiency), and therefore report the results for fertilization rate. However, the results did not change when seed set was used as the response variable.

We used the data from the pollen supplementation experiment to calculate a pollen limitation index: $L = 1 - (P_o / P_s)$, where P_o is the percent seed set of open-pollinated flowers or inflorescences, and P_s is the seed set of flowers that received supplemental pollination (Larson and Barrett 2000). Fruit set and seed set are equivalent in both species. Negative values, which indicate a higher seed set in natural than in pollen-supplemented flowers, were set to zero, as also done by Young and Young (1992) and Larson and Barrett (2000). Strong negative values are likely caused by experimental errors, such as damage to flowers during pollination, and very small negative values are likely caused by chance, when pollen limitation does not actually occur. The inclusion of negative values did not change the main results. The P value of the effect of the number of compatible inflorescences within a 2 m radius of *Armeria* increased from 0.03 to 0.05, but all other P -values for both *Armeria* and *Ranunculus* were within the same limits as before.

Pollen deposition in *Armeria*

To estimate the natural pollen deposition in *Armeria*, we collected two stigmas from the centers of three inflorescences of each individual ($n = 50$); i.e., a total of six stigmas per individual. The stigmas were collected from flowers in a late phase of anthesis, as indicated by the pale pink color of the corolla and the wilting of anthers, in the afternoon on two consecutive days (22–23 May), and we counted the pollen directly afterwards. Stigmas were stored separately in 24-well microtiter plates during transportation to the lab, where the stigmas were dissected and gently squeezed under a cover glass. We counted and identified pollen grains under a Leitz Laborlux 11 microscope and categorized them as (i) incompatible grains (i.e., pollen of the same morph type as the target individual), (ii) compatible grains (i.e., pollen from the other morph type), and (iii)

heterospecific pollen. Since the flowers were not emasculated, it was not possible to separate self-incompatible pollen from incompatible pollen from other individuals. Therefore, we only included compatible and heterospecific pollen grains in the analyses.

Inventory of the floral neighborhood

To assess the relationship between the floral neighborhood and the magnitude of pollen limitation of individuals, we counted the number of flowers within four concentric rings (0–0.5 m, 0.5–1 m, 1–2 m, and 2–3 m) around each supplementary-pollinated individual, and used these data to compute the flower densities within circles of radius 0.5, 1, 2, and 3 m. We monitored the densities of conspecific compatible and incompatible individuals and inflorescences of *Armeria*, and the densities of conspecific flowers and ramets of *Ranunculus*. We also counted the number of flower units (flowers or inflorescences) of other species within the neighborhoods, and the total number of inflorescences (*Armeria*; range = 6–80) or flowers (*Ranunculus*; range = 8–66) on the target individuals. Finally, we measured plant height and the height of the surrounding grass sward around each target individual of *Ranunculus*, but we did not do this for *Armeria*, which does not grow in tall grass vegetation. We used these data to compute the heights of the *Ranunculus* individuals relative to the height of the surrounding vegetation, since this could affect the detection of flowers by pollinators.

Observations of pollinators

To investigate the relationship between floral neighborhood and pollinator visitation to *Ranunculus*, we observed pollinator visits on nine sunny and calm days between 7 and 18 June (its peak flowering period). The observations were done on flowers within 1×1 m plots. Ten such plots were haphazardly chosen across a range of densities of *Ranunculus* flowers at each day of observation; thus, we observed 90 plots in total. This haphazard selection of plots could possibly have biased our data set, since the plots not were chosen by total randomization. We observed each plot only once, during a 6 min period. We identified each pollinator that entered the plot to the lowest possible taxa, and monitored the number of *Ranunculus* flowers visited by each pollinator. All insects that landed on or made contact with the sexual parts of the flowers were considered to be pollinators. After each observation period we counted the number of *Ranunculus* flowers and the number of flower units of other species within the plot and in a 0.5 m-wide strip around the plot. These data were used to calculate the number of arrivals in the plot, the mean number of flowers visited per pollinator, and the flower visitation rate per hour

(the total number of flowers visited in the plot during 6 min \times 10/total number of flowers in a plot).

Statistical analyses

We used randomized block mixed-model ANOVAs to analyze the effects of pollinator exclusion and pollen supplementation on the seed set in *Armeria* and the fertilization rate in *Ranunculus*. Individual was used as a random factor and treatment (bagging or supplemental pollination) as a fixed factor. In the analysis of pollen supplementation in *Ranunculus*, flowering time was included as an additional fixed factor because the abundance of pollinators may change over time and thereby affect pollen limitation. Flowering time was not added as a factor in the analysis of *Armeria* since the analyses were made at the inflorescence level and inflorescences did not differ much in flowering time. No transformations were needed in the analyses of the pollen supplementation experiment. In the analysis of pollinator exclusion, seed set and fertilization rate were arcsine transformed to fulfill the requirements of the ANOVA (Sokal and Rohlf 1997). Interaction terms with $P > 0.25$ were omitted from the final models (Underwood 1997).

We examined how the floral neighborhood affected pollen limitation and pollen deposition with multiple regressions or, if the residuals were not normally distributed, with generalized multiple regression models. Generalized regressions were corrected for overdispersion, had a Poisson distribution, and had log as the link function. We searched for the best model using best subset regressions (Neter et al. 1996), with P to enter = 0.05 and P to remove = 0.1. The effects of the predictor variables were analyzed separately for each radius, since strong correlations occurred among variables in the different radii (Pearson correlation coefficients ranged from 0.37 to 0.97; all were significant at $P < 0.05$). This correlation was expected, since the large circles included the data for the smaller circles. The utilization of four separate tests of the circles and the fact that larger circles included the data for the smaller circles could increase the Type 1 error rate. This can be solved by adjusting α using the Bonferroni correction. However, such corrections have been accused of being too conservative (Nakagawa 2004), and so we chose not to do this. To reduce the number of predictor variables, we used the density of conspecific flowers or inflorescences and not the density of individuals, since these were strongly correlated in both *Armeria* ($r = 0.81, 0.75, 0.80$, and 0.86 for a radius of 0.5, 1, 2, and 3 m, respectively, all significant at $P < 0.05$) and *Ranunculus* ($R = 0.73, 0.92, 0.96$ and 0.92 for a radius of 0.5, 1, 2, and 3 m, respectively, all significant at $P < 0.05$). The variables used in the initial models of pollen limitation and deposition of compatible pollen grains

in *Armeria* included the densities of compatible, incompatible and heterospecific inflorescences and the number of inflorescences on the target individual. The initial model used for the deposition of heterospecific pollen grains in *Armeria* included the densities of conspecific and heterospecific inflorescences and the number of inflorescences on the target individual. The variables used in the initial model for pollen limitation in *Ranunculus* included the densities of conspecific and heterospecific flowers or inflorescences, the number of flowers on the target plant, and the relative height of the target plant.

The effects of the conspecific and heterospecific flower densities on the number of pollinators arriving at the plots, the mean number of *Ranunculus* flowers visited per pollinator, and the floral visitation rate were investigated using multiple regressions. The best model was searched for using best subset regression (P to enter = 0.05 and P to remove = 0.1). The initial model included the flower densities of *Ranunculus acris* and *Stellaria graminea* and the inflorescence density of *Anthriscus sylvestris*. Since the flower/inflorescence densities of other species were very low, these data were not included in the model. All variables were log-transformed.

Results

Armeria was the most abundant flowering species in its area (mean number of inflorescences within the inventoried 3 m circles: 240.9), followed by *Scleranthus perennis* (mean inflorescences: 11.84), *Silene uniflora* (mean flowers: 7.16), *Cochlearia officinalis* (mean inflorescences: 7.04), and *Potentilla erecta* (mean flowers: 5.82). *Ranunculus* was the most common flowering species in its area (mean number of flowers within the inventoried 3 m circles: 1290.6), along with *Anthriscus sylvestris* (mean inflorescences: 89.6) and *Stellaria graminea* (mean flowers: 91.5). Less abundant flowering species included *Veronica chamaedrys* (mean inflorescences: 12.2), *Trifolium pratense* (mean inflorescences: 2.62), *Leucanthemum vulgare* (mean inflorescences: 0.65), *Lotus corniculata* (mean inflorescences: 0.3), and *Achillea millefolium* (mean inflorescences: 0.03).

Selfing

Bagged *Armeria* inflorescences and *Ranunculus* flowers produced no or very few seeds compared to open-pollinated ones (*Armeria* seed set: bagged = 0.04 ± 0.01 , $n = 10$, control = 0.690 ± 0.02 , $n = 10$, mean \pm SE, $F = 1031.6$, $df = 1, 34$, $P < 0.001$; *Ranunculus* fertilization rate: bagged = 0.06 ± 0.02 , $n = 25$, control = 0.74 ± 0.04 , $n = 25$, $F = 121.2$, $df = 1, 24$, $P < 0.001$).

Pollen limitation at the population level

Pollen limitation at the population level, as indicated by a significant effect of pollen supplementation on reproduction, occurred in *Armeria* (seed set, supplemental pollination: 0.64 ± 0.02 , $n = 38$, control: 0.47 ± 0.02 , $n = 38$), but not in *Ranunculus* (fertilization rate, supplemental pollination: 0.61 ± 0.01 , $n = 72$, control: 0.58 ± 0.01 , $n = 72$) (Table 1). In *Ranunculus* there was a significant effect of seed collection time on fertilization rate (Table 1). We therefore analyzed the effect of supplemental pollination on fertilization rates within seed collection time 2, the time when most seedheads were collected. We then obtained similar results to those obtained when all flowering times were analyzed together. The significant interaction between individual and treatment in *Armeria* shows that individuals respond differently to supplemental pollination; in other words that variations in pollen limitation occur among individuals in the same population (Table 1).

Pollen limitation of individuals

Histograms of individual pollen limitation indices showed a large variation in the extent of pollen limitation among *Armeria* individuals, whereas the variation was smaller among *Ranunculus* individuals—they showed no or very little pollen limitation (Fig. 1). A significant relationship between floral neighborhood and pollen limitation in *Armeria* occurred within the 2 and 3 m circles (Table 2). The best model included the densities of compatible and heterospecific flowers within the 3 m circle (Table 2) where pollen limitation was negatively related to the density of compatible flowers and positively related to density of heterospecific flowers (Fig. 2). In *Ranunculus*, no significant relationships were found between pollen limitation and floral neighborhood at any of the spatial scales investigated ($P > 0.05$ for all variables).

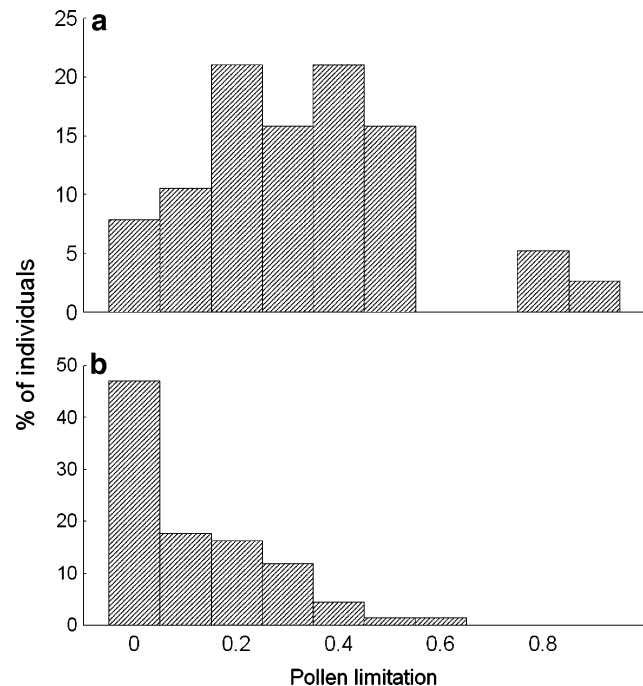


Fig. 1 Frequency histograms of individual pollen limitation in populations of **a** *Armeria maritima* spp. *maritima* and **b** *Ranunculus acris* spp. *acris*. A value of 0 indicates that the seed set is not constrained by pollen availability, and a value of 1 indicates that the seed set is completely constrained by pollen availability

Pollen deposition in *Armeria*

Deposition of compatible pollen grains increased with the number of inflorescences on the target individual, and this was consistent over the four radii (Table 3). For the 0.5 m radius, the deposition of compatible pollen was positively related to the density of compatible inflorescences and negatively related to the density of incompatible inflorescences (Table 3). The number of heterospecific pollen grains deposited on stigmas increased with the density of heterospecific flower units in the neighborhood, although this relationship only occurred within the 2 m radius (Table 3).

Table 1 Effect of pollen supplementation on seed set in *Armeria maritima* spp. *maritima* and fertilization rate in *Ranunculus acris* spp. *acris*, as investigated using a randomized block mixed-model ANOVA

Response variable	Source of variation	df	MS	F	P
Seed set in <i>Armeria</i>	Individual	37	0.15	7.12	0.001
	Treatment	1	1.42	67.35	0.001
	Individual \times treatment	37	0.02	1.81	0.009
	Error	120	0.01		
Fertilization rate in <i>Ranunculus</i>	Individual	71	0.35	10.13	0.001
	Treatment	1	0.08	2.5	0.11
	Seed collection time	4	1.03	38.34	0.001
	Individual \times treatment	71	0.03	1.27	0.076
	Error	620	0.027		

Table 2 Relationships between floral neighborhood and pollen limitation in *Armeria maritima* spp. *maritima*, investigated using multiple regressions with best subset regression

Response variable	Circle radius	Predictor variable	df	β	R^2
Pollen limitation	0.5	No significant predictor variables			
	1	No significant predictor variables			
	2	# Compatible inflorescences	Model: 2 Residual: 35	−0.4*	0.12
	3	# Compatible inflorescences	Model: 2	−0.39**	0.25
		# Heterospecific flowers	Residual: 35	0.37*	

The explanatory values of the models are indicated by the standardized regression coefficients (β) and the adjusted multiple coefficient of determination (R^2)

Only significant predictor variables are shown, and the following significance levels are indicated: * $P < 0.05$ and ** $P < 0.01$

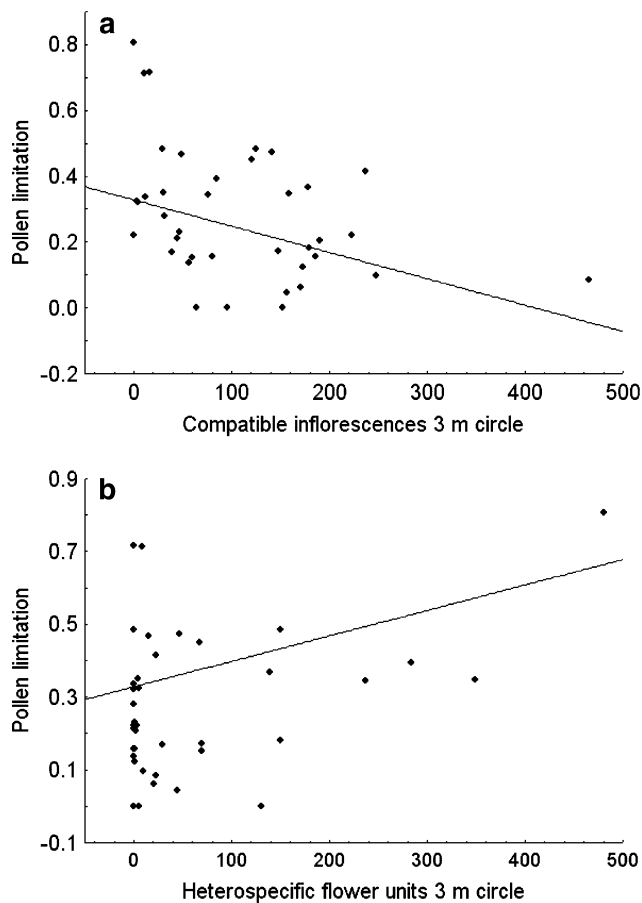


Fig. 2 The relationship between pollen limitation and **a** the number of compatible inflorescences and **b** the number of heterospecific flower units within a 3 m radius from the target individual in *Armeria maritima* spp. *maritima*. A pollen limitation value of 0 indicates that the seed set is not constrained by pollen availability, and a value of 1 indicates that the seed set is completely constrained by pollen availability. The regression lines are derived from the parameters of a multiple regression

Pollinator visits to *Ranunculus*

We recorded 227 visits to *Ranunculus*. The most frequent visitors were the hoverfly *Cheilosia* sp. (12% of visits) and

Empis sp. (10%), and four small unidentified solitary bee species (each ca. 10%). The number of pollinator arrivals at the plots was positively related to the density of *Ranunculus* flowers (range: 10–300 flowers per plot), and the mean number of *Ranunculus* flowers visited per pollinator was positively related to the density of *Ranunculus* flowers and the density of *Anthriscus* inflorescences (Table 4). Visitation rate to *Ranunculus* flowers was positively related to the density of *Anthriscus* inflorescences (range: 0–85 inflorescences per plot), and no other variables significantly explained the variation in the *Ranunculus* visitation rate (Table 4).

Discussion

Conspecific density and pollen limitation

The negative relationship between the density of compatible inflorescences and individual pollen limitation found in *Armeria* is in line with the results of three other within-population studies of pollen limitation and conspecific flower density (de Jong et al. 2005; Wagenius 2006; Zorn-Arnold and Howe 2007). All of these studies found negative relationships between conspecific flower density (measured either as conspecific density or as isolation of target individuals) and individual pollen limitation. Several within-population studies have associated conspecific flower density with other variables that are related to pollen limitation, such as seed/fruit production (Roll et al. 1997; Somanathan and Borges 2004; Pettersson 1997; Spigler and Chang 2008), pollen deposition and/or removal (Johnson et al. 2003; Duncan et al. 2004), stigma persistence (Wagenius et al. 2007), or seed/fruit production in combination with pollinator visits and/or pollen deposition (Aizen 1997; Bosch and Waser 1999, 2001; Caruso 1999; Caruso 2002; Kirchner et al. 2005). None of these studies have found a negative relationship between conspecific density and reproductive success (i.e., relationships were either positive or absent). These within-population studies suggest that the number of neighboring

Table 3 Relationships between floral neighborhood and pollen deposition in *Armeria maritima* spp. *maritima*, investigated using generalized multiple regression models with a Poisson distribution and log used as the link function

Response variable	Circle radius (m)	Predictor variable	Wald	Parameter estimate	df	P
Compatible pollen	0.5	# Inflorescences of target individual	5.2	0.02	1	0.022
		# Compatible flowers	5.2	0.02		0.021
		# Incompatible flowers	3.8	−0.02		0.049
	1	# Inflorescences of target individual	5.9	0.04	1	0.02
	2	# Inflorescences of target individual	4.5	0.03	1	0.03
Heterospecific pollen	3	# Inflorescences of target individual	4.3	0.03	1	0.04
	0.5	No significant predictor variables				
	1	No significant predictor variables				
	2	# Heterospecific flowers	4.8	0.006	1	0.028
	3	No significant predictor variables				

Only significant predictor variables are shown

Table 4 Relationship between pollinator visits to *Ranunculus acris* spp. *acris* and the densities of con- and heterospecific flowers

Response variable	Predictor variable	df	β	R^2
Arrivals at the plot	# <i>Ranunculus</i> flowers	Model: 2	0.30*	0.09
		Residual: 60		
Flowers visited per pollinator	# <i>Ranunculus</i> flowers	Model: 3	0.25*	0.14
	# <i>Anthriscus</i> inflorescences	Residual: 59	0.32*	
Visitation rate	# <i>Anthriscus</i> inflorescences	Model: 1	0.34**	0.11
		Residual: 88		

The explanatory values of the models are indicated by the standardized regression coefficients (β) and the adjusted multiple coefficient of determination (R^2)

Only significant predictor variables are shown: * $P < 0.05$, ** $P < 0.01$

conspecifics does not normally exceed the level where flower visitation rate decreases as a function of flower density due to competition for pollinators (Rathcke 1983). This is also supported by the majority of among-population studies of population density and reproductive success (Ghazoul 2005 and references therein). Thus, facilitation among conspecifics at the local scale seems to be the most common situation in natural populations.

Two different mechanisms could underlie such facilitation among conspecifics: an increase in pollinator visitation and/or an increase in the quality of each visit. First, it is commonly observed that dense patches of a flowering species are more attractive to pollinators than ones in which the flowering species is scarce (Kunin 1997a; Bosch and Waser 2001). Second, a higher visit quality can be caused by an increase in the amount of compatible pollen deposited on the stigmas, due to a higher availability of compatible pollen in the neighborhood (Stehlik et al. 2006), or by an increase in cross-pollination and a decrease in geitonogamic crosses if pollinators probe fewer flowers per plant and bypass more plants when plant density increases (Zimmerman and Pyke 1988; Cresswell 1997; Goverde et al. 2002). The positive relationship between compatible

inflorescences and deposition of compatible pollen in *Armeria* shows that a higher availability of compatible pollen in the neighborhood increases the transfer rate of compatible pollen, but we cannot tell if this is caused by increased visitation or increased quality of each visit. We found a negative relationship between the density of conspecific incompatible flowers and the deposition of compatible pollen, but this did not transfer into a negative effect on pollen limitation. This could be explained by either the negative effect of incompatible flowers being too small to affect pollen limitation, or by the conspecific incompatible flower density having a compensatory positive effect through increased pollinator attraction to the patch, which increases the likelihood of visitation for all conspecific flowers.

Heterospecific density and pollen limitation

The effect of local heterospecific density on plant reproductive success has been much less studied than the effect of conspecific density. However, some of the abovementioned within-population studies on conspecific flower density and reproduction also assessed the effects of heterospecific flower density (Caruso 1999, 2002; Johnson et al. 2003). Of

these, only [Johnson et al. \(2003\)](#) found an effect on plant reproductive success, and this was the special case of increased pollination success in a deceptive orchid, caused by co-occurring magnet plants. The positive relationship between the density of heterospecific flowers and pollen limitation found in our study should be approached with some caution, since removing the outlier point at ca. 500 heterospecific flower units from the analysis makes the relationship nonsignificant. We nevertheless think that this relationship deserves attention. A negative relationship between heterospecific flower density and reproduction could be the result of at least two processes: a decrease in the rate of visitation of *Armeria* flowers if heterospecific flowers are more attractive (e.g., [Chittka and Schürkens 2001](#); [Brown et al. 2002](#); [Totland et al. 2006](#)) and/or an increase in the transfer of heterospecific pollen if pollinators visit different species during foraging bouts (e.g., [Galen and Gregory 1989](#); [Caruso and Alfaro 2000](#); [Jakobsson et al. 2007](#)). The latter process could negatively affect reproduction through stigma clogging or chemical interference (e.g., [Galen and Gregory 1989](#); [Murphy 2000](#)). Unfortunately, no data are available on pollinator visitation for the *Armeria* system that could be used to test the hypothesis of competition for pollination. However, the deposition of heterospecific pollen increased with the density of heterospecific flowers, suggesting that at least the latter process could occur in our system.

Floral display size and pollen limitation

We found that the number of compatible pollen grains per stigma was positively related to the number of inflorescences of the target individuals. Individuals with larger floral displays often attract more pollinators per time unit (reviewed by [Ohashi and Yahara 1999](#)), whereas the relationship between floral display size and per flower visitation rate can be positive, negative, or nonexistent ([Grindeland et al. 2005](#) and references therein). Our result suggests that, in the *Armeria* population, the number of flowers visited per plant increased more than proportionally with display size. However, the effects of display size on pollen deposition did not translate into effects on pollen limitation.

The case of *Ranunculus*

For *Ranunculus*, we found that neighbors affected different aspects of pollinator visitation to individuals, but we did not find any relationship between the floral neighborhood and pollen limitation. This highlights the importance of investigating both visitation and its effects on reproduction in studies of pollinator facilitation/competition in plant species. The reason for a lack of relationship between the floral

neighborhood and pollen limitation was most likely the observed absence of pollen limitation at the population level, and the small variations in pollen limitation found among individuals. An absence of pollen limitation at the population level means that either the transfer of compatible pollen is sufficiently high even for the plants with lowest visitation rates, or that there are strong constraints imposed by nutrient availability that prevent individuals from responding to an increase in pollen availability ([Haig and Westoby 1988](#)). Strong constraints due to resource limitation are unlikely in our *Ranunculus* population because fertilization rates were fairly high (around 60%), and did not substantially differ from the final seed set (<5%).

The positive relationship found between arrivals at a plot and the density of *Ranunculus* flowers suggests that pollinators prefer patches with high flower density, and thereby confirms the findings of several other studies ([Feldman 2006](#) and references therein). This relationship did not, however, translate into an increased visitation rate in dense plots of *Ranunculus*, probably because visitation rate did not increase faster than the number of flowers ([Rathcke 1983](#); [Aizen 1997](#); [Bosch and Waser 2001](#)). Rate of visitations to *Ranunculus* flowers did, however, increase with the density of *Anthriscus sylvestris* inflorescences, providing support for the facilitation of pollinator attraction between coflowering species. Such facilitation between coflowering plant species has been predicted under certain circumstances ([Rathcke 1983](#); [Feldman et al. 2004](#)), but with the exception of nonrewarding plants pollinated by deceit, empirical evidence of it is still limited (but see [Schemske 1981](#); [Moeller 2004](#); [Ghazoul 2006](#)). Of the two coflowering species, *Anthriscus sylvestris* and *Stellaria graminea*, only *Anthriscus* affected rate of visitation to *Ranunculus*. The reason for this was probably that (in contrast to *Stellaria*) it was frequently visited and shared pollinators with *Ranunculus*.

Methodological concerns

Pollen limitation at the flower level does not automatically translate into pollen limitation at the individual level ([Zimmerman and Pyke 1988](#)), because an overestimation of pollen limitation may result if individuals allocate more resources to pollen-supplemented flowers at the expense of naturally pollinated flowers ([Ashman et al. 2004](#)). However, in a meta-analysis of pollen limitation studies, [Knight et al. \(2006\)](#) found that when pollen limitation was assessed between inflorescences (as in our study) and compared to the whole plant level, there was no significant difference in the magnitude of pollen limitation. Moreover, in our study we are interested in the relationship between pollen limitation and floral neighborhood, and even if there is a slight overestimation of pollen limitation we can still investigate

this relationship as long as the overestimation is of the same magnitude for all individuals (de Jong et al. 2005). The magnitude of overestimation could potentially be affected by plant size because resource allocation in small plants could be more pronounced than in large plants. However, we did not find any relationship between pollen limitation and the number of inflorescences in *Armeria* ($R^2 = 0.0004$, $P = 0.89$), or number of flowers ($R^2 = 0.03$, $P = 0.09$), or plant height ($R^2 = 0.2$, $P = 0.88$) in *Ranunculus*. This suggests that our method is adequate to test our hypothesis.

Spatial scale

The size of the floral neighborhood affecting the reproductive success of a particular species may depend on search behavior, foraging range and diet breadth of its pollinators (Ghazoul 2005). A priori assumptions about the size of the neighborhood (as we used in our study) are then necessary. We based the delimitation of our study on the facts that bumblebees (the main pollinator of *Armeria*) often limit their foraging flights to an area of only a few square meters, despite being able to move much further (e.g., Smithson and Macnair 1997), and that flies (one of the main pollinators of *Ranunculus*) move relatively short distances (up to a few meters) between plants (e.g., Widen and Widen 1990).

For *Armeria*, no relationship between flower density and pollen limitation occurred at the two smallest spatial scales. At the 2 m radius, flower density explained 10% of the pollen limitation, and the model that included flower density within the 3 m radius explained most of the variation in pollen limitation (25%). Thus, to understand how the floral neighborhood influences pollen limitation in our study species, we had to monitor areas that were larger than 1 m in radius around the target individuals. This differs from a study by Roll et al. (1997), who found that including the density of conspecifics at distances over 1 m did not increase the amount of explained variation in reproductive success in *Lesquerella fendleri*. In another study, Spigler and Chang (2008) found that reproductive success of *Sabatia angularis* decreased with the density of conspecific neighbors for a 1 m radius around target plants, but increased for a 4 m radius. Although these studies are not fully comparable with ours, since we estimated pollen limitation and Roll et al. (1997) and Spigler and Chang (2008) estimated seed production, the results suggest that the relevant spatial scale at which conspecific density affects reproductive success may differ among plant species. Such species specificity can be caused by differences in pollinator assemblages, which in turn differ in foraging strategies and the method of perceiving floral resources (e.g., Sih and Baltus 1987; Ghazoul 2005). It could be also caused by differences in the sensitivity to outcrossing and/or inbreeding depression of the plant species (Waser and Price 1989; Byers and Waller 1999).

Contrary to what could be expected from our findings of significant relationships between pollen limitation and floral neighborhood in *Armeria* at the 2 and 3 m radii, a relationship between deposition of compatible pollen and floral neighborhood was only found within the 0.5 m radius. This discrepancy might be explained by the difference in the scales at which pollinators perceive the flowers and choose a patch for foraging, and the scale at which the movements within a local patch occur. Flowers at the larger scale may contribute to attracting pollinators to the patch, increasing the number of visitors to the patch and thereby also to the target individual. However, as predicted by the optimal foraging theory, once the pollinators are within a patch they tend to move among the closest flowers in order to reduce search times and maximize their energy gain per unit time invested in foraging (Stephens and Krebs 1986). Therefore, the likelihood that a visiting pollinator would carry compatible pollen should be mostly dependent on the floral neighborhood in the immediate vicinity of the target individuals. The reason why the small scale effect on pollen deposition did not influence pollen limitation may be that closely situated flowers are closely related to the target individual and therefore provide pollen of lower quality (e.g., Robertson and Ulappa 2004). In contrast to our results, Spigler and Chang (2008) found a correspondence between pollen deposition and reproductive success, with both increasing with plant density at the 4 m radius.

The deposition of heterospecific pollen grains increased with the density of heterospecific flower units within the 2 m radius, and was thus in closer agreement with the spatial scale at which the effect of heterospecific flowers on pollen limitation occurred (the 3 m radius). A possible explanation for this could be that bumblebees (the main pollinators of *Armeria*) normally show flower-constancy behavior, limiting their visitations to one or few species during foraging bouts (e.g., Waser 1986). Therefore, their visits to other neighboring plant species may occur only occasionally and by chance, and not as the result of foraging in several species at a time.

Implications of our study

Our study showed that the pollination successes of individual plants may depend not only on their phenotypical characteristics and the local density of conspecifics, but also on the local densities of other species. From an evolutionary point of view, this may have important consequences for the selection of floral traits (Smithson 2001). From an ecological point of view, this is of interest for the conservation and management of plants. Our finding of facilitation among conspecifics in a pollen-limited plant supports the notion that decreases in density of plants will have negative effects on their reproductive success and drive the species into negative feedbacks, possibly resulting in the local loss of the

species. Limited pollen dispersal and pollen limitation are ubiquitous, especially in the context of habitat fragmentation (Burd 1994; Aizen et al. 2002; Knight 2005), and it is therefore of great importance to understand the processes and mechanisms behind this. Many studies have investigated the effects of mean density and population size on pollination (reviewed in Kunin 1997b). However, the individual-based measure of pollen limitation used in our study relates more closely to pollen limitation theory, which predicts ecological and evolutionary causes and consequences for individual plants, not populations (Ashman et al. 2004). It therefore provides an important link between **pollen limitation theory and studies of the effects of landscape fragmentation on plant species persistence**. Our study also shows that investigations on multiple spatial scales are useful for increasing our knowledge of the scales at which processes occur for different types of species, and are necessary in order to understand the real causes of pollen limitation.

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