

Effects of local abundance on pollination and reproduction in the narrow endemic endangered species *Delphinium bolosii* (Ranunculaceae)

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Abstract

Effects of local abundance, in terms of plot size (number of individuals) and purity (relative abundance), on pollination interactions were studied by examining the quantity and quality of pollinator services and subsequent seed set in the narrow endemic, endangered and self-compatible species *Delphinium bolosii*. The most frequent visitors were Lepidoptera (mainly *Macroglossum stellatarum* and two species of *Gonopteryx*) and Hymenoptera (small bees). Differences in relative frequency of visits to plants were observed between populations, possibly due to the intrinsic local insect fauna. Plots with high numbers of individuals attracted more pollinators, so plants from these plots were visited more often. At the individual level, no effect of size was detected but visitation rates were significantly different between the two studied populations. The same pattern was observed for stigmatic pollen loads and seed set. The effects of purity were assessed by comparing pure plots of *D. bolosii* with a mixed plot that also included the neighbor species *Rubus ulmifolius*, an invasive shrub. Although interspecific competition for pollinator visits was observed, conspecific pollen deposition did not vary between pure and mixed plots. However, negative effects on reproductive traits such as higher rates of seed abortion (50%) and a lower S/O index (viable seeds/ovules) were detected in individuals from the mixed plot. These results may be helpful for developing conservation measures, especially in one of the populations, where *R. ulmifolius* is abundant due to nearby abandoned agricultural land.

Key words: competition, conservation, pollinator services, purity, seed set, size, stigmatic pollen loads.

Resum. Efectes de l'abundància local en la pol·linització i reproducció de l'espècie endèmica de distribució restringida i amenaçada *Delphinium bolosii* (Ranunculaceae)

Els efectes de l'abundància local, en termes de mida de parcel·la (nombre d'individus) i puresa (abundància relativa), en les interaccions de pol·linització s'han estudiat mitjançant l'avaluació de la quantitat i la qualitat dels serveis de pol·linització i la consegüent pro-

ducció de llavors en l'espècie endèmica de distribució restringida, amenaçada i autocompatible *Delphinium bolosii*. Els visitants més freqüents van ser lepidòpters (especialment *Macroglossum stellatarum* i dues espècies de *Gonopteryx*) i himenòpters (abelles petites). Les diferències en la freqüència relativa de visites a les plantes que es van observar entre poblacions són possiblement degudes a l'abundància local intrínseca de fauna d'insectes. Les parcel·les amb un nombre elevat d'individus van atreure més pol·linitzadors, de manera que les seves plantes van ser visitades més sovint. A escala individual, no es va detectar efecte de la mida; però la proporció de visites va ser significativament diferent entre les dues poblacions estudiades. El mateix patró es va trobar per a les càrregues pol·líniques estigmàtiques i la producció de llavors. Els efectes de la puresa es van analitzar comparant les parcel·les pures que només contenien *D. bolosii* amb una parcel·la mixta, que contenia altres plantes com l'espècie veïna *Rubus ulmifolius*, un arbust invasor. Malgrat que es va observar competència interespecífica per les visites dels pol·linitzadors, el pol·len conespecífic dipositat no variava entre parcel·les pures i mixtes. No obstant això, es van detectar efectes negatius en alguns paràmetres reproductius com una major proporció d'avortament de les llavors (50%) i una davallada de l'índex S/O (llavors viables/prímordis seminals) dels individus en les parcel·les mixtes. Aquests resultats poden ser útils en el desenvolupament de mesures de conservació, especialment en una de les poblacions, en què *R. ulmifolius* és abundant degut a l'abandonament dels terrenys de conreu propers.

Paraules clau: competència, conservació, serveis de pol·linització, puresa, producció de llavors, mida, càrregues pol·líniques estigmàtiques.

Introduction

In wild species populations, local abundance can vary influenced by ecological relationships and stochastic events. Nevertheless lately anthropogenic disturbances have been producing a dramatic decline in species abundance (Saunders et al., 1991). Local abundance is defined as a complex issue related to population size (number of individuals), population density (spatial distance between neighboring conspecifics), and population purity (the relative abundance of the species) (Kunin, 1997a). Variation in any of these parameters is expected to produce significant changes in ecological interactions (such as pollination) and population dynamics. Particularly sensitive to abundance variation and its consequences are narrow endemic species, which usually occur in small populations and specialized habitats (Kirchner et al., 2005).

The negative effects of reduction of population size have been widely documented in both pollination interactions (Jennersten, 1988; Aizen & Feinsinger, 1994) and the reproductive success of species (Ågren, 1996; Bosch et al., 2002). Larger plant populations are likely to attract more pollinators resulting in higher visitation rates (Kunin, 1997a, and references therein), whereas small populations may suffer from insufficient pollinator visits, and possibly pollen limitation and lower seed set (Kwak et al., 1998; Ramsey & Vaughton, 2000; Wolf & Harrison, 2001).

Besides population size, density may also affect plant-pollinator interactions and hence plant reproduction (Feinsinger et al., 1986; Kunin, 1992; Kunin, 1993; Roll et al., 1997). For example, low densities of flowering plants are frequently related to low pollination and visitation rates, increased intraspecific competition

for limited pollinator services and hence, low reproductive success (Platt et al., 1974; Waser, 1983; Bosch & Waser, 1999; but see Zimmerman, 1980; Aizen, 1997). Spatial distribution of plants is also important for the genetic flow within a population, since pollinator foraging behavior may change depending on the distance between neighbor plants, promoting inbreeding or out-cross pollination (van Treuren et al., 1993; de Jong et al., 1993; Karron et al., 1995).

Furthermore, a sparse population might be intermixed with other plants. This mix may have a facilitative effect by increasing total floral density and hence visitation rates, or an adverse effect by enhancing interspecific competition for pollinator services and resources (Sih & Baltus, 1987; Bjerknes et al., 2007). Besides affecting the quantity of visits, density variation in mixed populations can change pollinator service quality (Thomson, 1981; Caruso, 1999). While a high correlation between population size and density is reported in the wild (van Treuren et al., 1993; Ågren, 1996), experimental manipulations of spatial distribution have also been performed to assess the effect of each factor independently (Campbell, 1985; van Treuren et al., 1993; Kunin, 1993; Bosch & Waser, 2001; Mustajärvi et al., 2001). Although this methodology provides a better control of conditions, it is not always recommendable since it supposes a high risk of damage to the populations, especially in endangered species for which not much experimental manipulation is feasible due to their conservation status (Forsyth, 2003).

Delphinium bolosii (Ranunculaceae) provides a good case study to explore local abundance effects on pollination in narrow endemic plants because it is a very rare and threatened species with a reduced number of individuals, and with complex floral morphology suggesting potential pollinator dependence. It is included in the *Catálogo Nacional de Especies Amenazadas* (The Spanish Catalogue of Endangered Species, BOE, 1990) and listed as endangered in the Red Book of Vascular Spanish flora (Bañares et al., 2003). The major threats to *D. bolosii* are related to habitat loss derived from rural activities and agricultural abandoning. This latter has led to the development of invasive shrubs, such as *Rubus ulmifolius*, in one of the two known populations for this species.

In this study, we examine how local abundance variation, in terms of size and purity, affects pollination and reproductive success in *D. bolosii*. The specific questions addressed were: (1) Is the quantity of pollinator visits affected either by the number of *D. bolosii* individuals or by the presence of *R. ulmifolius*? (2) Is the quality of pollination visits, expressed as stigmatic pollen load, affected by such variation in local abundance? (3) Is seed set also affected?

Material and methods

Study species

Delphinium bolosii C. Blanché and Molero is endemic to Catalonia (North-eastern Iberian Peninsula) and only two populations are known at present (Figure 1). In 2002, the population located near Rubió de Baix, La Noguera (LNO) - UTM 31TCG34 - included 2,500 individuals growing in few lines of plants occupying

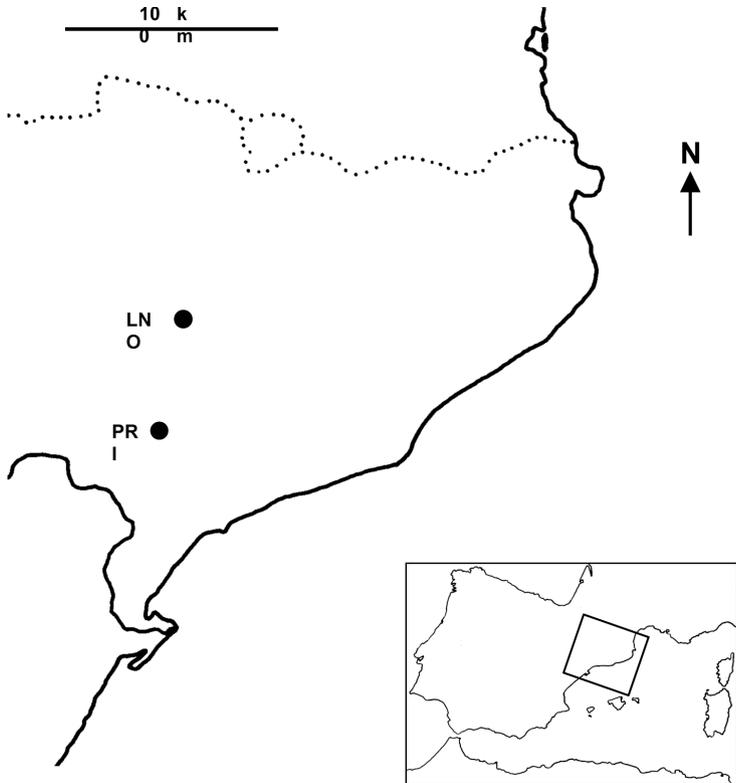


Figure 1. Distribution area and location of the studied populations of *D. bolosii*. ● LNO: La Noguera, Rubió de Baix ● PRI: Priorat, Ulldemolins.

ledges on a calcareous cliff facing north over the river Segre (290 m elevation). In this location, *D. bolosii* is accompanied by *Antirrhinum molle* L., *Buxus sempervirens* L., *Coronilla emerus* L., *Jasminum fruticans* L. and *Orlaya grandiflora* L., among others. The other population is located about 60 km away, near Ulldemolins, Priorat (PRI) - UTM 31TCF27. It contains approximately 1,000 individuals growing over a hedge in a narrow ravine through a *Quercus rotundifolia* forest, accompanied mainly by *Rubus ulmifolius*, a co-flowering invasive shrub that grows intermixed with *D. bolosii* (630 m elevation). Other frequent species in this population are *Saponaria ocymoides*, *Globularia vulgaris*, *Hypericum perforatum*, *Bryonia cretica* and *Psoralea bituminosa*. Demographic studies on *D. bolosii* have shown that approximately only 10% of the total number of individuals flower each year (unpubl. data), reducing notably the amount of reproductive plants per population.

Table 1. Area and number of individuals of *Delphinium bolosii* in the large, small and mixed plots established at La Noguera (LNO) and Priorat (PRI) populations.

Relative Abundance	Plot size	Area (m ²)	LNO	Area (m ²)	PRI
Pure	Large	4	13	4	7
	Small	4	5	4	2
Mixed			–	4	7

Delphinium bolosii is a self-compatible, rhizomatous, perennial larkspur. It grows forming basal rosettes of dissected leaves that usually sprout in February. During the blooming period (June- July), a reduced number of rosettes develop a raceme of 1.5-2 m with 15-100 purple-blue zygomorphic flowers. Flowers are protandrous and pollination is entomophilous. Fruits produce 30-45 black seeds of 2-3 mm, distributed across three follicles. At the end of the flowering period, the aerial part of the plant becomes dry and the rhizomes remain dormant, forming a rootstock bank. Also, vegetative multiplication by rhizome fragmentation is possible.

Pollinator visitation

Pollinator observations were conducted in June 2002 on three different days at each site. To quantify pollinator visitation rates, we established in each population two plots of 2 × 2 m² with different numbers of individuals, representing its natural abundance variation. Given the endangered condition of this species, the reduced number of flowering plants, and the fact that most of them grow in inaccessible places, we were not able to establish more plots. “Large” plots in LNO and PRI population consisted of 13 and 7 plants, respectively; while “small” plots of 5 and 2 plants for the same populations. In the PRI population, we established an additional “mixed” large plot with 7 *D. bolosii* individuals and several *Rubus ulmifolius* that covered approximately 70% of plot area. A single mixed plot was established in PRI population since other potential mixed plots of *D. bolosii* were completely covered by *R. ulmifolius*. Plots were selected to be as isolated as possible and each plant within each plot was individually labeled (Table 1).

We observed pollinators in the 5 plots of *D. bolosii* during a series of 15 minute-period (one census), spread evenly between 10.00 and 20.00 (GMT). Observations of the different plots at a given site were made simultaneously by different observers. In total, we carried out 148 censuses, 72 in LNO (35 in the small plot and 37 in the large one) and 76 in PRI (32 in the small plot, 32 in the large plot and 12 in the mixed plot). In each census we recorded the species identity of each pollinator, its foraging behavior, the number of approaches to a plot (number of different insects that visit at least one flower) and the total number of plants and flowers visited per plot. During each observation session, the total number of available flowers per plot was recorded to determine whether a relationship exists be-

tween the number of flowers visited per census and the number of flowers displayed. The unrecognized visitors were caught for further identification.

Pollen deposition

Pollinator service quality was estimated as pollen loads deposited on stigmas of already pollinated flowers. For this reason, we used senescent flowers, i.e. those whose petals have started turning brownish and falling down. We collected the stigmas of 20 flowers from different plants in each plot. The three bilobate stigmas per flower were mounted in basic fuchsin gel on a microscope slide (Kearns & Inouye, 1993) and quantities of conspecific and heterospecific pollen were counted at 100–400x magnification. Heterospecific pollen was identified by comparison to a pollen reference collection from the surrounding flowered plants, which are also potential competitors.

Reproductive success

Reproductive success was measured as seed set from 20 fruits randomly collected from each plot. We counted all expanded seeds and undeveloped ovules per fruit. In order to compare seed set between plants, the ratio of viable vs. aborted seeds was calculated and expressed as a reproductive index of seed/ovule (S/O).

Data analysis

We used two-way ANOVAs to test the effects of size and population on various pollination parameters recorded during the 15 min census (number of pollinator approaches to a plot, number of plants and flowers visited per plot, number of flowers visited per displayed flowers in a plot, number of pollinator approaches per plant, proportion of plants visited in a plot, number of flowers visited per plant, and number of flowers visited per displayed flowers per plant), pollen loads (total pollen load, conspecific pollen load and heterospecific pollen load), and seed set (developed seeds, aborted seeds, total seeds and S/O ratio). We considered a mixed model, with size as a fixed factor and population as a random factor. Non-significant interactions were omitted in the final analysis (Sokal & Rohlf, 1995). When an interaction was significant, fixed effects were tested over the interaction. The effect of purity on pollinator visits, stigmatic pollen loads and seed set was assessed using one-way ANOVAs, comparing the mixed and pure large plots at the PRI site. All ANOVAs were of Type III.

Results

Pollinator visitation

Delphinium bolosii attracted a wide spectrum of visitors, with slight differences in their diversity between populations, but important variation in visitation frequency (Table 2).

Table 2. Pollinator visits observed on *Delphinium bolosii* at La Noguera (LNO) and Priorat (PRI) populations.

Visitors	LNO			PRI		
	Appr. (%) ^a	Pl. (%) ^b	Flo. (%) ^c	Appr. (%) ^a	Pl. (%) ^b	Flo. (%) ^c
HYMENOPTERA						
Anthophoridae						
<i>Amegilla fasciata</i>				0.16	0.20	0.09
Apidae						
<i>Apis mellifera</i>	1.02	0.50	0.19	0.79	0.60	0.60
<i>Bombus pascuorum</i>	8.69	4.28	5.17			
<i>Bombus terrestris</i>	0.85	0.56	0.44	0.47	0.40	0.44
Eumenidae						
<i>Alastor atropos</i>	3.92	1.57	0.56	16.40	14.18	10.42
Halictidae						
<i>Lassioglossum</i> sp.	6.99	2.64	0.57	37.69	30.80	21.00
LEPIDOPTERA						
Lycaenidae						
<i>Satyrrium ilicis</i>				0.31	0.20	0.19
Nymphalidae						
<i>Cynthia cardui</i>	0.68	0.50	0.14	0.31	0.20	0.12
Papilionidae						
<i>Papilio machaon</i>	1.70	1.29	0.93			
Pieridae						
<i>Artogeia rapae</i>	0.17	0.05	0.01			
<i>Gonopteryx cleopatra</i>	1.87	0.73	0.20	15.93	17.83	22.30
<i>Gonopteryx rhamni</i>	1.19	0.39	0.06	17.98	21.68	25.80
Satyridae						
<i>Lasiomata megera</i>	0.17	0.05	0.07	0.15	0.10	0.03
<i>Pyronia bathseba</i>	1.87	0.84	0.20	0.15	0.10	0.09
Sphingidae						
<i>Macroglossum stellatarum</i>	67.63	84.72	89.96	4.41	8.20	12.20
Zygaenidae						
<i>Zygaena</i> sp.				0.15	0.10	0.09
DIPTERA						
Bombyliidae						
<i>Bombylius</i> sp.	2.21	1.35	1.24	4.41	4.86	6.29
Syrphidae						
<i>Eristalis tenax</i>	0.17	0.11	0.06	0.47	0.40	0.22
HETEROPTERA						
Pentatomidae						
<i>Graphosoma</i> sp.	0.17	0.11	0.11			
COLEOPTERA						
Scarabaeidae						
<i>Oxythirea funesta</i>	0.35	0.17	0.01	0.16	0.10	0.06

Note: In all plots studied, total recorded approaches were 1221, total visited plants were 2760 and total visited flowers were 14547 during the observation period.

^a "Appr." is the percentage of total number of approaches to a plot by each pollinator taxon.

^b "Pl." is the percentage of the total number of plants visited.

^c "Flo." is the percentage of the total number of flowers visited.

Table 3. ANOVAs of effects of size and population on pollinator visitation parameters per plot, all per 15-min observation period of *Delphinium bolosii*.

Source	Approaches/plant			Total no. of plants visited/plot			Total no. of flowers visited/plot			No. of flowers visited/ no. of flowers opened		
	df	SS	F	df	SS	F	df	SS	F	df	SS	F
Size	1	696.5	11.87	1	7931.1	3.64	1	216190	1.81	1	1.55	6.19 *
Population	1	319.9	26.61***	1	8010.9	92.84***	1	529123	154.88***	1	14.82	59.00***
Size × Population	1	58.7	4.88*	1	2179.2	25.25***	1	119553	34.99***		–	
Error	132	1586.7		132	11389.9		132	450954		133	33.42	

Note: All data that did not have significant interactions were removed from the analysis. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 4. ANOVAs of effects of size and population on pollinator visitation parameters per plant, all per 15-min observation period of *Delphinium bolosii*.

Source	Approaches/plot			Total no. of plants visited/plot			Total no. of flowers visited/ no. of flowers opened					
	df	SS	F	df	SS	F	df	SS	F			
Size	1	0.0008	1.08	1	0.0021	0.69	1	0.1570	1.16	1	0.0014	50.80***
Population	1	0.0044	5.95*	1	0.0129	4.11*	1	4.0996	30.44***	1	0.0000	1.74
Size × Population		–			–			–			–	
Error	133	0.0994		133	0.4318		133	17.910		133	0.0036	

Note: All data that did not have significant interactions were removed from the analysis. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

A total of 587 insects were recorded visiting plants in the studied plots in the LNO population. Almost 70% of these visitors were *Macroglossum stellatarum* (Lepidoptera), around 20% bees (Hymenoptera) and the remaining 10% a miscellaneous of insects, including diverse species of Lepidoptera and Diptera (mainly *Bombylius* sp.). Meanwhile, a total of 634 insects were recorded in the studied plots in the PRI population. Approximately 50% were small bees (Hymenoptera), mainly *Lassioglossum* sp. (Halictidae) and *Alastor atropos* (Eumenidae); and 35% were Lepidoptera (*Gonopteryx cleopatra* and *G. rhamni*). In comparison to the LNO population, only a few approaches (4%) were made by *M. stellatarum*, and similar rates were observed for *Bombylius* sp. (Table 2).

In the PRI population, honeybees (*Apis mellifera*) were frequent but preferred to visit *Rubus ulmifolius* flowers, rather than *D. bolosii* ones. On the other hand, we observed in both populations the presence of nectar robbers, bumble bees (*Bombus terrestris* and *B. pasquorum*) and small bees (*A. atropos*), acting as primary and secondary nectar robbers, respectively. In general, visitation rates of small bees, including *A. atropos* and *Lassioglossum* sp., were probably undervalued due to systematic observational error as their tiny body size (about 12 mm) made identification difficult. For this reason values for these species were not taken into account for further ANOVA analyses. We also did not consider Heteroptera and Coleoptera species because their visits were accidental, not contributing to pollination.

Macroglossum stellatarum, *Gonopteryx* sp. and *Bombylius* sp. visited a high number of plants and flowers in each bout, whereas the remaining species visited very few plants and flowers (Table 2). Approximately 90% (LNO) and 50% (PRI) of visits to flowers were made by *M. stellatarum* and *Gonopteryx* sp. Considering all except the excluded visits (see Methods), the number of plants of *D. bolosii* determined the number of approaches to plots in LNO and PRI populations (Table 3). Thus large plots were approximately two times more visited than small ones in both populations (Figure 2), with this size effect being much more evident in the LNO population (Table 3). The same trend was found when considering the total number of plants and flowers visited per plot. However, when open flower availability was considered, small plots were more visited in both populations.

At the plant level no plot size effect was detected on the number of approaches, nor on the proportion of visited plants in a plot or the number of visited flowers (Table 4). On average, plants and flowers of both large and small plots received quite similar number of pollinator visits. Nevertheless, differences in visitation rates were detected between populations (Table 4). At LNO, higher rates of approaches were recorded when number of individuals was factored in, but more plants and flowers per plant were visited at PRI. Conversely, when considering flower availability, there was a significant effect of plot size being the plants of small plots more visited, but no differences between populations were observed (Figure 3).

The presence of *R. ulmifolius* had negative effects on pollination parameters at PRI since the number of pollinators' approaches to the mixed plots was lower

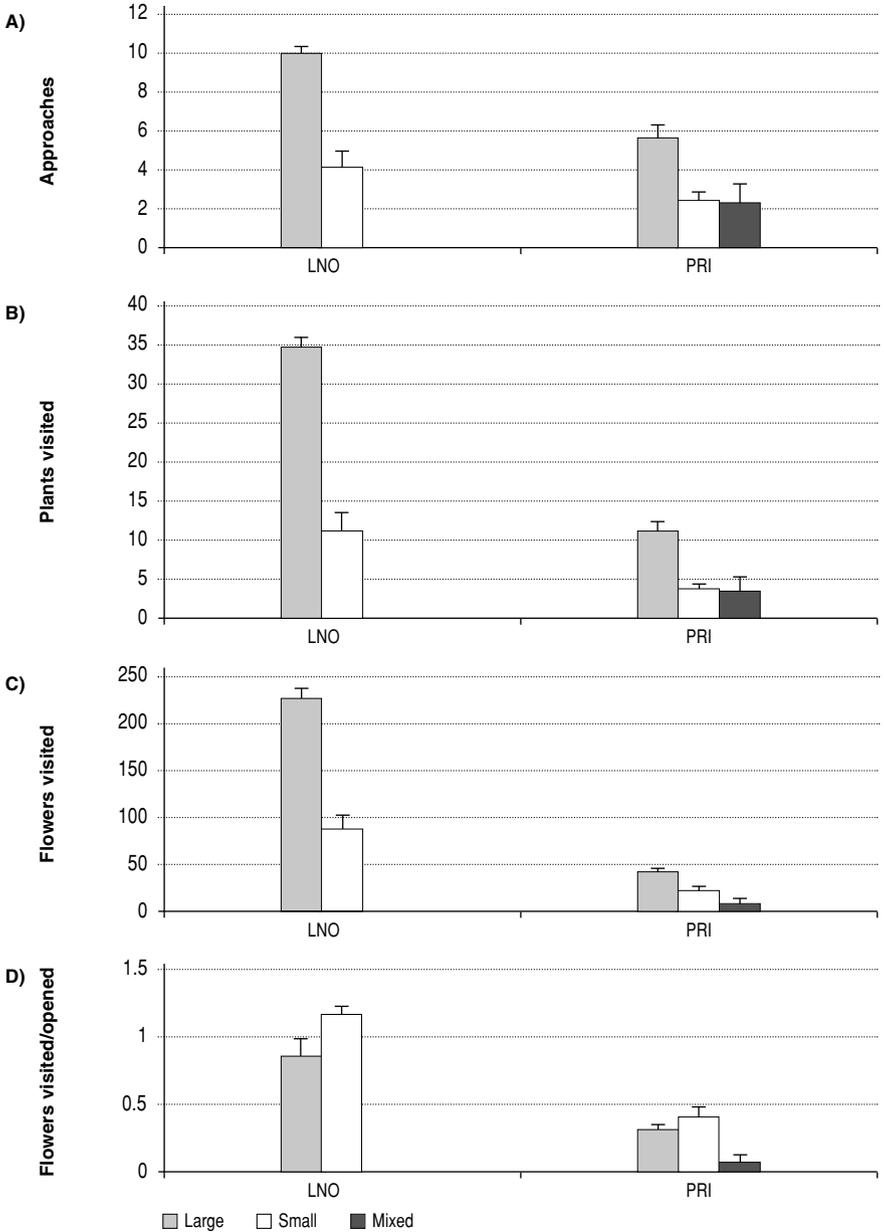


Figure 2. Pollinator visitation per 15-min census in the large (■), the small (□) and the mixed plots (■) of the two studied populations of *D. bolosi* (LNO and PRI): A) number of approaches to a plot, B) number of plants visited per plot, C) number of flowers visited per plot, and D) number of flowers visited per open flowers in a plot. Each bar represents mean \pm standard error. Conventions follow Figure 1.

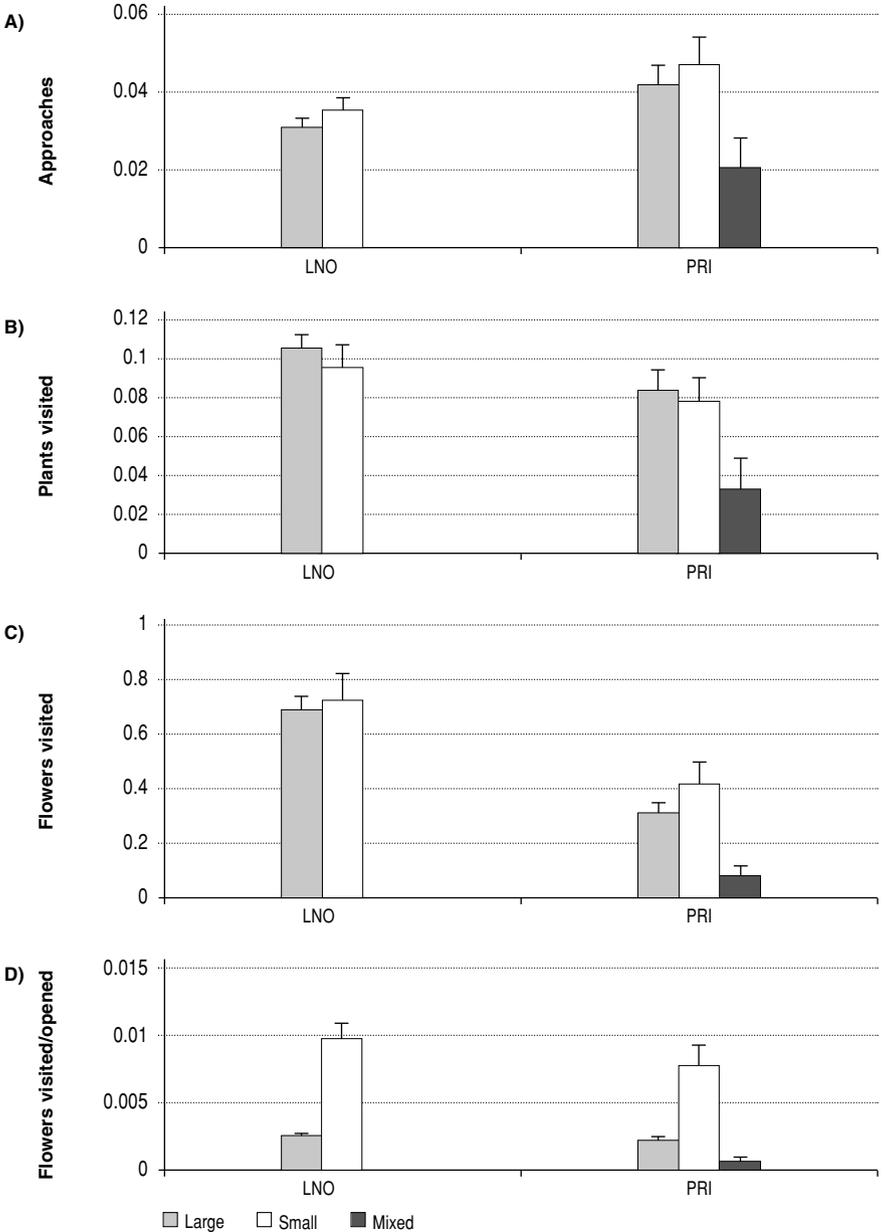


Figure 3. Pollinator visitation per 15-min census in the large (■), the small (□) and the mixed plots (■) of the two studied populations of *D. bolosi* (LNO and PRI): A) number of approaches to a plant, B) proportion of plants visited in a plot, C) number of flowers visited per plant, and D) number of flowers visited per open flowers per plant. Each bar represents mean ± standard error. Conventions follow Figure 1.

Table 5. ANOVAs of effects of size and population on pollen deposition on stigmas of *Delphinium bolosii*.

Source	Conspecific			Heterospecific			Total		
	df	SS	F	df	SS	F	df	SS	F
Size	1	11688.6	0.49	1	9.11	0.07	1	11045.0	0.44
Population	1	593574	24.73***	1	2.81	0.02	1	596160	23.87***
Size x Population	—	—	—	—	—	—	—	—	—
Error	77	1.84×10^6	—	77	9766.6	—	77	1.92×10^6	—

Note: All data that did not have significant interactions were removed from the analysis. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 6. Origin of the stigmatic pollen loads in the studied populations of *Delphinium bolosii*.

Species	Pollen	
	LNO	PRI
<i>Delphinium bolosii</i>	96.80	92.60
<i>Hypericum perforatum</i>	2.22	0.80
<i>Sedum</i> sp.	—	2.39
<i>Rubus ulmifolius</i>	—	1.93
<i>Saponaria ocyroides</i>	—	0.54
<i>Sarcocapnos eneaphylla</i>	0.45	—
Others	0.53	1.74

Note: Values are expressed as percentage in descending order. Total pollen grains counted were 14320 in LNO and 10186 in PRI.

than the observed for pure plots ($F_{[1,42]}=7.49$, $P=0.009$). Also, the presence of *R. ulmifolius* plants within *D. bososii* plots reduced the overall visitation rate ($F_{[1,42]}=10.49$, $P=0.002$), as well as the number of flowers visited ($F_{[1,42]}=16.84$, $P=0.000$) and the ratio of flowers visited in relation to the available ones ($F_{[1,42]}=14.61$, $P=0.001$) (Figure 2). Visitation frequency to *D. bososii* individuals was also negatively affected in the mixed plot. Total pollinator approaches per plant ($F_{[1,42]}=5.26$, $P = 0.027$), the number of visited plants ($F_{[1,42]} = 7.95$, $P = 0.007$), the number of visited flowers per plant ($F_{[1,42]} = 14.61$, $P = 0.001$) and the number of flowers visited in relation to the available ones per plant ($F_{[1,42]} = 12.20$, $P = 0.001$), were all lower in the mixed plot, with *R. ulmifolius*, than in the pure one with only *D. bososii* plants (Figure 3).

Pollen deposition

The mean number of pollen grains of all species deposited on *D. bososii* stigmas was approximately 245 (range 19-871, $N = 100$). The numbers were similar in large and small plots, but differed significantly between sites (Table 5, Figure 4); with flowers in the LNO population receiving more pollen grains. The same trend was observed when considering only pollen from conspecifics, with stigmas in the LNO population receiving twice as many pollen grains as those in the PRI population (Table 5, Figure 4). The amount of heterospecific pollen on *D. bososii* stigmas was much lower (3% of all pollen received in the LNO population and 8% in the PRI population). The most common heterospecific pollen grains on *D. bososii* stigmas included *Hypericum perforatum*, *Sedum* sp. and *Rubus ulmifolius*, among others (Table 6). No differences in heterospecific pollen deposition between

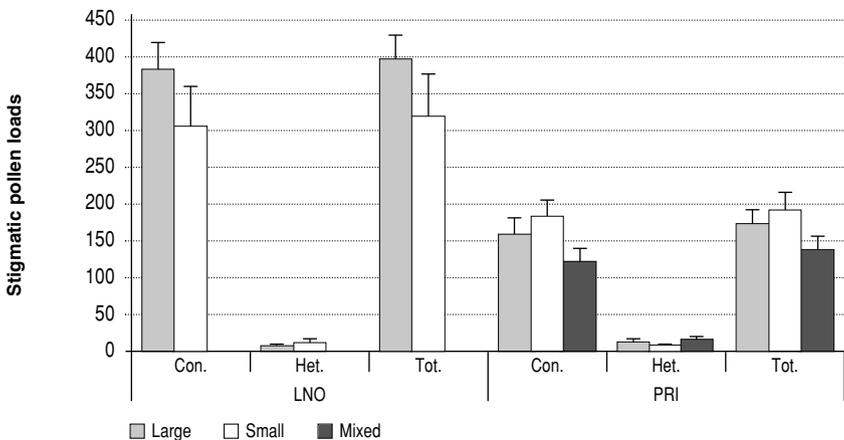


Figure 4. Pollen deposition on the stigmas of plants from large and small plots in the two studied populations of *D. bososii*. “Con.”: Conspecific pollen loads, “Het.”: heterospecific pollen loads, and “Tot.”: total pollen loads. Each bar represents mean \pm standard error. Conventions follow Figure 1.

large and small plots were detected. No effects of purity on total pollen loads ($F_{[1,38]} = 1.55$, $P = 0.221$), conspecific pollen loads ($F_{[1,38]} = 1.84$, $P = 0.183$), or even heterospecific pollen loads ($F_{[1,38]} = 0.15$, $P = 0.704$) were observed when comparing the mixed and pure plots at PRI.

Seed set

The overall mean seed set per flower was 23.8 (range 0-45, $N = 100$) and each flower produced 38.7 ovules on average (range 15-51). The production of well developed seeds and the S/O index did not show any significant variation between large and small plots (Figure 5, Table 7). Also these reproductive parameters were very similar between populations, regardless of the differences detected in the frequency of pollinator visitation. However, plants from PRI produced more ovules per fruit and thereby they had a higher seed abortion rate than the LNO population (Figure 5, Table 6). Within the PRI population, mixing had no effect on seed set ($F_{[1,38]} = 1.84$, $P = 0.183$) or ovule production ($F_{[1,38]} = 1.84$, $P = 0.102$), but produced a higher rate of seed abortion ($F_{[1,38]} = 8.74$, $P = 0.005$) and lower S/O index ($F_{[1,38]} = 4.74$, $P = 0.036$).

Discussion

The field observations showed that the most frequent visitors recorded for *D. bolosii*, in particular Hymenoptera and Lepidoptera, differed greatly between populations, probably due to the characteristic micro-fauna and intrinsic abundance at each site (Rathcke, 1983; Feisinger et al., 1991; Talavera et al., 2001). Bosch et al. (1998) reported similar visitation rates for the main pollinators in 1992 and 1993, with

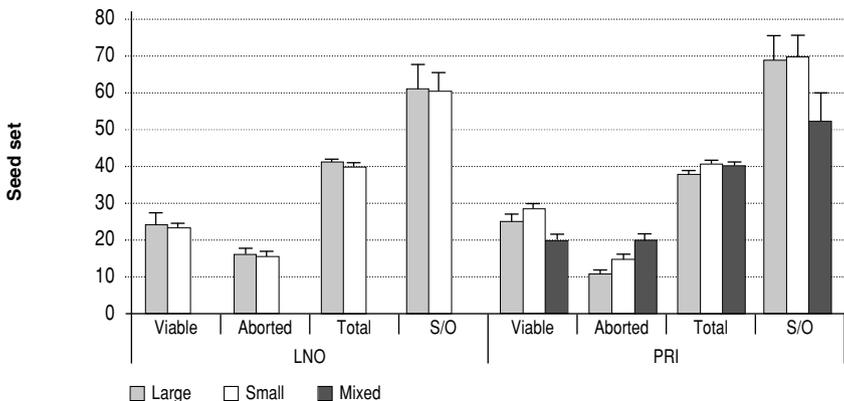


Figure 5. Seed set from large and small plots in the two studied populations of *D. bolosii*. “Viable”: well-developed seeds, “Aborted”: not developed ovules, and “Total”: total ovules produced per plant, “S/O”: percentage of seeds per ovule. Each bar represents mean \pm standard error. Conventions follow Figure 1.

Table 7. ANOVAs of effects of size and population on seed set of *Delphinium bolosii*.

Source	Seeds developed			Aborted seeds			Total			S/O		
	df	SS	F	df	SS	F	df	SS	F	df	F	
Size	1	18.05	0.23	1	15.31	0.29	1	66.61	0.26	1	0.000	0.000
Population	1	0.8	0.01	1	324.01	6.12*	1	292.61	6.90*	1	0.083	2.410
Size x Population				1	255.61	6.03*						
Error	77	6021.2		77	4078.2		76	3222.7		77	2.64	

Note: All data that did not have significant interactions were removed from the analysis. Statistical significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

a slightly higher diversity of Hymenoptera, Lepidoptera and Diptera. These spatial and temporal fluctuations in visitor quantity may also be dependent on the environmental conditions (Cruden, 1972; Herrera, 1988; Herrera, 1995; Molano-Flores et al., 1999).

Our results suggest that the local number of conspecific plants of *D. bosonii* affected pollinator visitation rates, but not pollen loads or final seed set in both populations. Due to the benefits of mutual attraction, plots with more conspecific plants enjoyed higher visitation rates to *D. bosonii* plants and flowers, outweighing the cost of intraspecific competition for pollinator services (Schaal, 1978; Kunin & Iwasa, 1996; Kunin, 1997b; Bosch & Waser, 1999). However, in certain cases, the probability of a given plant being visited decreases proportionally with the number of individuals present (optimal foraging theory, Charnov, 1976; Pyke, 1984). Such competition for pollinators would have been evident on analysis of the results at the plant level, but no differences were detected. Pollinators probably preferred to visit several different plants close to each other than invest more energy in flying relatively long distances to find another plant in small plots (Feinsinger, 1983; Kunin, 1992; Bosch & Waser, 2001; Mustajärvi et al., 2001; but see Talavera et al., 2001). Similarly, when considering the total number of opened flowers of this species either at the plot or at the plant level, our results suggest that rates of visitation to individual flowers are negatively related to the number of available flowers because competition for pollinator services is likely to be increased in conditions of high abundance (Steven et al., 2003).

Besides intraspecific competition, our results give insights into a negative effect of interspecific competition on *D. bosonii* pollination. Visitation rates were reduced in the mixed plot, even at the plant level. In the PRI population, the presence of *Rubus ulmifolius* hindered Lepidoptera pollinators visiting *D. bosonii* flowers, due to the former plant's prickles. In addition, as has been reported for other species of *Rubus* (Oostermeijer et al., 1998), *R. ulmifolius* competes for pollinators since some visitors to *D. bosonii* carry over *R. ulmifolius* pollen (Bosch, 1999) and it exerts a strong attraction on *Apis mellifera*. This marked preference of honeybees for *R. ulmifolius* could explain the relatively low percentage of *Rubus* pollen found in *D. bosonii* flowers. Possibly the additive effect of interspecific and intraspecific competition for pollinator services explains the low rates of overall approaches and visits recorded in the PRI population in comparison to LNO. However, the influence of *R. ulmifolius* on *D. bosonii* pollination should be assessed during the entire blooming period, since variation in visitation rates across the flowering season in mixed plots had been previously reported for other species (Sih & Baltus, 1987).

Local number of plants of *D. bosonii* did not affect stigmatic pollen loads, which was expected as no significant size effect was observed for pollinator services at the plant level. But even for plants in the mixed plot, which were the least visited, no differences in pollen loads were detected. This lack of variation in the mixed plots may be related to two nonexclusive reasons. First, differences in pollinator effectiveness in carrying conspecific pollen could compensate for low visitation rates. However, most of *D. bosonii*'s floral visitors have been described as

generalists (Bosch et al., 1998) and efficient pollination by generalist visitors is expected to fail in poor levels of density and purity (Aizen & Feinsinger, 1994; Kunin, 1997a). Second, self-pollination might have increased stigmatic pollen loads (Molano-Flores et al., 1999). Despite *D. bolosii* being protandrous and presenting herkogamy, Bosch et al. (1998) reported spontaneous self-fertilization favored by the large amount of stamens (30-36) and pollen production (in the range 120,000-160,000 pollen grains per flower). Also, self-pollination could have been promoted by the behavior of the small bees (*Lassioglossum* sp.), frequent visitors who were usually observed walking around the stamens carrying high amounts of pollen stuck to their body.

As seed set depends, in part, on pollen deposition, no variation in viable seed production related to local number of plants was expected. Other studies on the effect of density and size in seed production differentiate between species with self-incompatible reproductive systems (*Brassica kaber* and *Palicourea* sp., Kunin, 1997b) and self-compatible ones (*Senecio jacobaea* and *Besleria triflora*, Feinsinger et al., 1991; *Lychnis viscaria*, Mustajärvi et al., 2001). In the first group, low density and size resulted in a low quantity of seeds being produced, while in self-compatible species there was no effect on seed quantity, despite differences in the frequencies of visits. In experimental assays under greenhouse conditions, *D. bolosii* produced approximately 20% of its seed set by self-pollination (Bosch et al., 1998). Therefore, *D. bolosii*, a self-compatible species with a non-strict protandry, does not seem to require highly efficient pollinator services to set seeds, compensating for any possible pollen deficit and maintaining invariable seed production regardless of the number of individuals. However, selfing can affect the genetic diversity levels of the species (Bosch et al., 1998; but see Orellana et al., 2007).

Despite similar stigmatic pollen loads, the relatively high seed abortion rate and the reduction of the S/O fraction for the mixed plot could show that interspecific competition is not just for pollinator services but also for resources that affect the development of available ovules in the PRI population. Nevertheless, from the point of view of conservation of this species, all possible effects of *R. ulmifolius* on *D. bolosii* should be explored before drawing any conclusion, because there may be some beneficial aspects. It seems that this invasive shrub, which grows after agricultural land is abandoned, forms a physical barrier that protects *D. bolosii* seedlings and rosettes from possible herbivory and human damage. Moreover we have evidence that *D. bolosii* is very sensitive to high temperatures and dryness, and *R. ulmifolius* could help to maintain humidity and shadiness.

In short, the consequences of local number of individuals in *D. bolosii* reproduction are influenced in a complex manner by its own breeding system, as well as pollinator and resource competition. We did not detect a substantial effect of the local number of plants on the studied parameters of reproduction; however, we identified potential detrimental consequences of interspecific competition on pollination and reproductive success for *D. bolosii*. Since other studies have demonstrated that long term studies are important for detecting these effects on plant reproduction (Molano-Flores & Hendrix, 1999), further research is needed to elucidate abundance dependent relationships for this species.

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References

- Ågren, J. 1996. Population size, pollinator limitation and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* 77: 1779-1790.
- Aizen, M.A. 1997. Influence of local floral density and sex ratio on pollen receipt and seed output: empirical and experimental results in dichogamous *Alstroemeria aurea* (Alstroemeriaceae). *Oecol.* 111: 404-412.
- Aizen, M.A.; Feinsinger, P. 1994. Habitat fragmentation, native insect pollinators, and feral honeybees in Argentine Chaco Serrano. *Ecol. Appl.* 4: 378-392.
- Bañares, A.; Blanca, G.; Güemes, J.; Moreno, J. C.; Ortiz, S. (eds.) 2003. Atlas y Libro Rojo de la Flora Vasculare Amenazada de España. Dirección General de la Conservación de la Naturaleza, Madrid.
- Bjerknes, A.L.; Totland, Ø.; Hegland, S. J.; Nielsen, A. 2007. Do alien plant invasions really affect pollination success in native plant species? *Biol. Conserv.* 138: 1-12.
- B.O.E. 1990. Catálogo Nacional de Especies Amenazadas [Real Decreto 439/1990, de 30 de marzo de 1990]. B.O.E. 5 de abril de 1990.
- Bosch, M. 1999. Biologia de la reproducció de la tribu Delphinieae a la Mediterrània occidental. Institut d'Estudis Catalans, Arxius de la Secció de Ciències 120. Barcelona.
- Bosch, M.; Simon, J.; Molero, J.; Blanché, C. 1998. Reproductive biology, genetic variation and conservation of the rare endemic dysploid *Delphinium bolosii* (Ranunculaceae). *Biol. Conserv.* 86: 57-66.
- Bosch, M.; Waser, N.M. 1999. Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* (Ranunculaceae). *Amer. J. Bot.* 86: 871-879.
- Bosch, M.; Waser, N.M. 2001. Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. *Oecol.* 126: 76-83.
- Bosch, M.; Simon, J.; Rovira, A.M.; Molero, J.; Blanché, C. 2002. Pollination ecology of the pre-Pyrenean endemic *Petrocoptis montsiciana* (Caryophyllaceae): effects of population size. *Biol. J. Lin. Soc.* 76: 79-90.
- Campbell, D.R. 1985. Pollinator sharing and seed set of *Stellaria pubera*: Competition for pollination. *Ecology* 66: 544-553.
- Caruso, C.M. 1999. Pollination of *Ipomopsis aggregata* (Polemoniaceae): Effects of intra- vs. interspecific competition. *Amer. J. Bot.* 86: 663-668.
- Charnov, E.L. 1976. Optimal foraging, the marginal value theorem. *Theor. Pop. Biol.* 9: 129-136.
- Cruden, R.W. 1972. Pollinators in high-elevation ecosystems: relative effectiveness of birds and bees. *Science* 176: 1439-1440.
- De Jong, T.J.; Waser, N.M.; Klinkhamer, P.G.L. 1993. Geitonogamy: the neglected side of selfing. *Trends Ecol. Evol.* 8: 321-325.

- Feinsinger, P. 1983. Coevolution and pollination. *In*: Futuyma D. J. and Slatkin M. (eds.). Coevolution. Sinauer Associated Publishers Sunderland, Massachusetts. pp. 282-292.
- Feinsinger, P.; Murray, K.G.; Kinsman, S.; Busby, W.H. 1986. Floral neighborhood and pollination success in four hummingbird-pollinated cloud forest plant species. *Ecology* 67: 449-464.
- Feinsinger P.; Tiebout III H.M.; Young B.E. 1991. Do tropical bird-pollinated plants exhibit density-dependent interactions? Field experiments. *Ecology* 72: 1953-1963.
- Forsyth, S. 2003. Density—dependent seed set in the Haleakala silversword: evidence for an Allee effect. *Oecol.* 136: 551-557.
- Herrera, C.M. 1995. Microclimate and individual variation in pollinators: Flowering plants are more than their flowers. *Ecology* 76: 1516-1524.
- Herrera, J. 1988. Variation in mutualism: the spatio-temporal mosaic of a pollinator assemblage. *Biol. J. Linn. Soc.* 76: 274-287.
- Jennersten, O. 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. *Conserv. Biol.* 2: 359-366.
- Karron, J.D.; Thumser N.N.; Tucker R.; Hessenauer A.J. 1995. The influence of population density on outcrossing rates in *Mimulus ringens*. *Heredity* 75: 175-180.
- Kearns, C.A.; Inouye, D.W. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado.
- Kirchner, F.; Luijten, S.H.; Imbert, E.; Riba, M.; Mayol, M.; González-Martínez, S.C.; Mignot, A.; Colas, B. 2005. Effects of local density on insect visitation and fertilization success in the narrow-endemic *Centaurea corymbosa* (Asteraceae). *Oikos* 111: 130-142.
- Kunin, W.E. 1992. Density and reproductive success in wild populations of *Diplotaxis erucoides* (Brassicaceae). *Oecol.* 91: 129-133.
- Kunin, W.E. 1993. Sex and the single mustard: population density and pollinator behavior effects on seed set. *Ecology* 74: 2145-2160.
- Kunin, W.E. 1997a. Population biology and rarity: on the complexity of density dependence in insect-plant interactions. *In*: Kunin W.E. and Gaston K.J. (eds.). The biology of rarity. Chapman and Hall Press, New York. pp. 150-169.
- Kunin, W.E. 1997b. Population size and density effects in pollination: pollination foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *J. Ecol.* 85: 225-234.
- Kunin, W.E.; Iwasa, Y. 1996. Pollinator foraging strategies in mixed floral arrays: density effects and floral constancy. *Theor. Pop. Biol.* 49: 232-263.
- Kwak, M.M.; Velterop, O.; van Andel, J. 1998. Pollen and gene flow in fragmented habitats. *Appl. Veg. Sci.* 1: 37-54.
- Molano-Flores, B.; Hendrix, S. 1999. The effects of population size and density on the reproductive output of *Anemone canedensis* (Ranunculaceae). *Int. J. Plant Sci.* 160: 759-766.
- Molano-Flores, B.; Hendrix, S.D.; Heard, S.B. 1999. The effect of population size on stigma load, fruit set, and seed set in *Allium stellatum* Ker. (Liliaceae). *Int. J. Plant Sci.* 160: 753-757.
- Mustajärvi, K.; Siikamäki, P.; Rytönen, S.; Lamni, A. 2001. Consequences of plant population size and density for plant-pollinator interactions and plant performance. *J. Ecol.* 89: 80-87.
- Orellana, M.R.; López-Pujol, J.; Blanché, C.; Bosch, M. 2007. Genetic diversity in the endangered dysploid larkspur *Delphinium bolosii* and its close diploid relatives in the series *Fissa* of the Western Mediterranean area. *Biol. J. Linn. Soc.* 92: 773-784.

- Oostermeijer, G.; Luijten, S.; Klenová, Z.V.; Den Nijs, H. 1998. Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L. *Conserv. Biol.* 12: 1042-1053.
- Pyke, G.H. 1984. Optimal foraging theory: a critical view. *Ann. Rev. Ecol. Syst.* 15: 523-575.
- Platt, W.J.; Hill, G.R.; Clark, S. 1974. Seed production in prairie legume (*Astragalus canadensis* L.): interactions between pollination, predispersal seed predation and plant density. *Oecol.* 17: 55-63.
- Ramsey, M.; Vaughton, G. 2000. Pollen quality limits seed set in *Burchardia umbellata* (Colchicaceae). *Amer. J. Bot.* 87: 845-852.
- Rathcke, B.J. 1983. Competition and facilitation among plants for pollination. In: L. Real (ed.). *Pollination Biology*. Academic Press, London, pp. 305-329.
- Roll, J.; Mitchell, R.J.; Cabin, R.J.; Marshall, D.L. 1997. Reproductive success increases with local density of conspecifics in a Desert Mustard (*Lesquerella fendleri*). *Conserv. Biol.* 11: 738-746.
- Saunders, D.A.; Hobbs, R.J.; Margules, C.R. 1991. Biological consequences of ecosystem fragmentation: a review. *Conserv. Biol.* 5: 18-32.
- Schaal, B.A. 1978. Density dependent foraging on *Liatrix pycnostachya*. *Evolution* 32: 452-454.
- Sih, A.; Baltus, M.S. 1987. Patch size, pollinator behaviour and pollinator limitation in catnip. *Ecology* 68: 1679-1690.
- Sokal, R.R.; Rolf, F.J. 1995. *Biometry*. W. H. Freeman, New York.
- Steven, J.C.; Rooney, T.P.; Boyle, O.D.; Waller, D.M. 2003. Density-dependent pollinator visitation and self-incompatibility in upper Great Lakes populations of *Trillium grandiflorum*. *J. Torrey Bot. Soc.* 130: 23-39.
- Talavera, S.; Bastida, F.; Ortiz, P.L.; Arista, M. 2001. Pollinator attendance and reproductive success in *Cistus libanotis* L. (Cistaceae). *International Journal of Plant Sci.* 162: 343-352.
- Thomson, J.D. 1981. Field measures of flower constancy in bumblebees. *Amer. Midl. Natur.* 105: 377-380.
- Van Treuren, R.; Bijlsma, R.; Ouborg, N.J.; Van Desde, R.W. 1993. The effects of population size and plant density on outcrossing rates in locally endangered *Salvia pratensis*. *Evolution* 47: 1094-1104.
- Waser, N.M. 1983. Competition for pollination and floral character differences among sympatric plant species: a review of evidence. In: Jones C.E. and Little R.J. (eds.). *Handbook of Experimental Pollination Biology*. Van Nostrand Reinhold Press, New York. pp. 277-293.
- Wolf, A.; Harrison S. 2001. Effects of habitat size and patch isolation on reproductive success of the serpentine morning glory. *Conserv. Biol.* 15: 111-121.
- Zimmerman, M. 1980. Reproduction in *Polemonium*: competition for pollinators. *Ecology* 61: 497-501.