

# Pollen limitation in a narrow endemic plant: geographical variation and driving factors

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**Abstract** Pollen limitation may have important consequences for the reproduction and abundance of plant species. It may be especially harmful to endangered and endemic plants with small populations. In this study, we quantify the effect of pollen limitation on seed production and seedling emergence in an endangered narrow endemic crucifer, *Erysimum popovii*. We conducted a pollen addition experiment across the entire geographic distribution of the species, and explored the effect of pollinator assemblage, plant population size and density, and other habitat variables on pollen limitation intensity in 13 populations. We supplemented flowers in 20 plants per population with allogamous pollen. To account for potential resource reallocation, we used two types of control untreated flowers: internal control flowers from the same individual as the supplemented flowers, and external control flowers from other individuals. Our results indicate that *E. popovii* is

pollen-limited in most of the populations studied, but only through seed production, since pollen supplementation did not enhance seedling emergence. Beefly abundance was associated with among-population differences in pollen limitation intensity. Populations in which beeflies were more abundant were less pollen-limited. In contrast, the abundance of other flower visitors, such as large bees or butterflies, was not associated with pollen limitation. Annual rainfall and bare soil cover were associated with the intensity of pollen limitation across populations.

**Keywords** Beefly · Pollination · Plant reproductive ecology · *Erysimum* · Endangered plant

## Introduction

Plant reproduction may be limited by the availability of resources (Suzuki 2000; Griffin and Barrett 2002), inadequate pollen receipt (Ashman et al. 2004; Knight et al. 2005; Aizen and Harder 2007), or a combination of both factors (Haig and Westoby 1988). Pollen limitation appears to be common across Angiosperms (Ashman et al. 2004; Knight et al. 2005). According to recent reviews, between 62 and 73 % of all insect-pollinated plants show evidence of inadequate pollen receipt (Ashman et al. 2004; García-Camacho and Totland 2009).

Pollen limitation may be caused by a decrease in pollen quantity or quality (Aizen and Harder 2007; Harder and Aizen 2010). Pollen quantity limitation occurs when pollinators are scarce or ineffective (depositing low amounts of pollen grains per visit) (Johnston 1991; Gómez et al. 2010). Pollen quality limitation occurs when pollinators deposit incompatible pollen, self-pollen, or pollen from closely related individuals, which may produce inbreeding

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depression and seed quality reduction (Herrera 1987; Pflugshaupt et al. 2002). Consequently, the potential effects of pollen limitation on reproductive success should ideally be measured in each of the plant's life-cycle reproductive stages (Ehrlén and Eriksson 1995). Yet, as noted by Knight et al. (2005), most studies only examine the consequences of pollen limitation on fruit and seed set, and few studies measure potential effects on post-dispersal stages (Ehrlén and Eriksson 1995; Price et al. 2008; Gómez et al. 2010).

The consequences of pollen limitation may be dramatic for plants. Reproductive failure due to pollen limitation may lessen population growth and long-term viability, resulting in declines in population size (Eriksson and Jakobsson 1998; Knight et al. 2005). Thus, pollen limitation may strongly influence the distribution and abundance of plant species and populations (Eriksson and Jakobsson 1998). Severe and consistent pollen-limitation may even trigger local extinctions in fragmented or heterogeneous habitats harboring small populations (Aguirre and Dirzo 2008; Hill et al. 2008). Reproductive failure due to pollen limitation is likely to be stronger in small, isolated populations than in large, well-connected populations (Aizen and Feinsinger 1994). This is especially important for the persistence of endemic plants, which have restricted distribution areas, and usually show lower fertility than their widespread congeners (Lavergne et al. 2004). Exploring the importance of pollen limitation for the reproduction of narrow endemic plants living in fragmented habitats is thus essential to understand and predict the consequences of the anthropogenic-mediated pollinator crisis on the conservation of plant populations (Allen-Wardell et al. 1998; Vamosi et al. 2006; Eckert et al. 2010).

In animal-pollinated plants, insufficient pollen deposition or deposition of low-quality pollen is mostly caused by pollinator assemblage characteristics, such as pollinator abundance, diversity, and identity. Many studies have found pollen limitation to be associated with decreased pollinator abundance (Baker et al. 2000; Cunningham 2000; Cosacov et al. 2008; Gonzalez-Varo et al. 2009; Gómez et al. 2010). In addition, pollen limitation may be related to decreased pollinator diversity (Gómez et al. 2010). This relationship appears when the plant's probability of being visited by effective pollen vectors increases with pollinator diversity (Perfectti et al. 2009). Pollen limitation is also related to the identity of pollinators, as different flower visitors differ in pollinating effectiveness and may have contrasting effects on plant fitness (Klein et al. 2003; Gómez et al. 2007; Ne'eman et al. 2010). In addition to pollinator assemblage traits, some characteristics of the plant population itself may influence pollen limitation intensity directly or indirectly. One example of direct effects is strong fragmentation, which may result in genetic impoverishment, thus setting a limit to the quality

of the pollen deposited, irrespective of pollinator community (Byers 1995). Indirect effects occur when plant population characteristics modify pollinator composition and behavior. For example, plant population size, habitat fragmentation, co-occurring flower composition, and altitude influence the community and activity of flower-visiting insects, and therefore the intensity of pollen limitation (Totland 2001; Gonzalez-Varo et al. 2009; Jakobsson et al. 2009).

The general goal of this study is to describe geographical variation of pollen limitation on seed and seedling production in an endangered Mediterranean endemic herb, *Erysimum popovii*, and to explore the potential factors influencing pollen limitation. Our specific objectives are: (1) to determine the geographic variation in pollen limitation throughout the entire distribution area of the species; (2) to establish at what stage, pre-dispersal (seed production) or post-dispersal (emergence rate), is pollen limitation more intense; (3) to study the association between flower visitor community and pollen limitation; and (4) to study the association between population characteristics (size, density of co-flowering species, habitat composition) and pollen limitation.

## Materials and methods

### Study system

*Erysimum popovii* Rothm. (Brassicaceae) is a narrow endemic plant from southeastern Spain, categorized as "Near Threatened" in the Red List of Andalusia Vascular Flora (Cabezudo et al. 2005). The species inhabits rocky areas and shrubland gaps in montane areas from 900 to 2,000 m. a.s.l. It is a biennial to perennial monocarpic herb, usually producing tens of flowers (mean  $\pm$  SD = 37.6  $\pm$  17.9 flowers; Table 1) on a variable number of reproductive stalks. Flowers are hermaphroditic and slightly protandrous, with four bright purple petals (Blanca et al. 1992, 2009). Flower lifespan is 1–2 days, depending on environmental and pollination conditions (authors' personal observation). Like other *Erysimum* species (Gómez 2005), *E. popovii* is self-compatible, but requires pollen vectors for full seed set (plants from which pollinators are excluded produce seeds, but in low numbers, authors' personal observation).

The study was conducted in 21 *Erysimum popovii* populations located in the Baetic Mountain range (Granada and Jaen provinces, southern Spain; Table 1), spanning the entire geographic range of the species (Fig. 1). Maximum distance between populations was 80 km. The area has a characteristic Mediterranean climate with cool wet winters and warm dry summers.

**Table 1** Location and characteristics of the 21 *E. popovii* populations studied

<i>E. popovii</i> populations	Region	Coordinates (UTM)	Elevation (m a.s.l.)	Population size (no. of <i>E. popovii</i> plants)	Density ( <i>E. popovii</i> plants/m <sup>2</sup> )	Flowers per plant <sup>a</sup>	Habitat	Mean annual temp. (°C)	Accumulated annual precipitation (mm)
01	Mencal, Granada	0487836 4155063	982	600	0.015	29.4 ± 19.08	Scrub	13.9	493.5
02	S. Arana, Granada	0451977 4128858	1,255	340	0.011	29.78 ± 12.61	Grassland + rocks	12.9	579.6
03	S. Nevada, Granada	0456751 4109531	1,596	280	0.037	26.8 ± 16.16	Scrub	10.7	510.2
04	La Peza, Granada	0467701 4118300	1503	300	0.073	21.27 ± 9.13	Grassland + rocks	11.8	577.0
05	S. Jabalcuz, Jaen	0425545 4177799	1,321	420	0.197	39.27 ± 34.34	Scree	12.1	725.6
07	S Mágina, Jaen	0453888 4176684	2,000	500	0.135	29.87 ± 28.42	Scrub	9.2	549.6
08	S. Jabalcuz, Jaen	0425519 4177931	1,324	400	0.173	21.8 ± 12.51	Pine forest	12.5	726.7
09	La Peza, Granada	0472834 4119460	1,693	130	0.085	37.23 ± 35.84	Grassland	10.4	609.1
10	S. Jabalcuz, Jaen	0424122 4177754	1,060	450	0.064	79.5 ± 77.45	Scree	13.5	737.3
13	S. Arana, Granada	0454337 4131901	1,738	370	0.087	29.97 ± 25.11	Grassland + rocks	9.9	642.4
14	S. Jabalcuz, Jaen	0426894 4180210	970	120	0.025	77.8 ± 116.95	Grassland + rocks	14.2	689.0
15	S. Jabalcuz, Jaen	0425148 4179387	1,080	650	0.062	69.27 ± 48.81	Scrub	13.9	648.0
16	S. Jabalcuz, Jaen	0425152 4179487	1,060	150	0.038	52 ± 54.09	Pine forest	13.8	640.5
19	Mencal, Granada	0483826 4151106	1,188	420	0.246	31.33 ± 27.37	Grassland	12.4	496.7
20	S. Arana, Granada	0451048 4130531	1,270	350	0.069	34.4 ± 21.86	Scrub	12.6	640.6
21	S. Arana, Granada	0452839 4131133	1,498	280	0.113	37.17 ± 20.63	Grassland	11.1	675.5
22	S. Arana, Granada	0453676 4128236	1,380	620	0.069	33.57 ± 19.36	Grassland	11.5	569.7
23	La Peza, Granada	0472949 4119278	1,720	750	0.556	41.37 ± 25.75	Grassland	10.3	602.6
27	S. Nevada, Granada	0457430 4109448	1,486	340	0.176	19.33 ± 11.50	Grassland	11.2	540.6
28	S Mágina, Jaen	0455789 4176686	1,661	1,650	0.193	32.87 ± 21.29	Grassland	10.9	559.4
30	S Mágina, Jaen	0453436 4176812	1,898	2,050	0.133	15.23 ± 6.388	Grassland	9.4	582.7

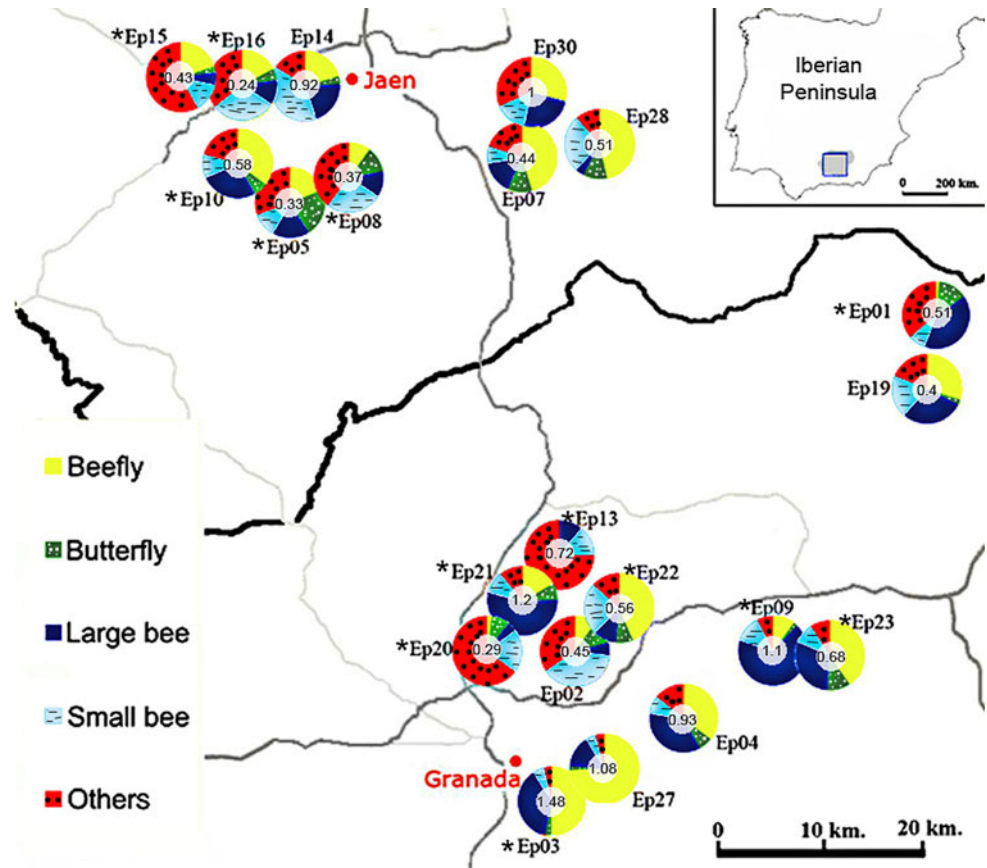
<sup>a</sup> Mean ± SD

### Pollen addition experiment

We conducted a pollen-supplementation experiment in the spring of 2009. In each of the 21 populations, we labeled 50 plants of average size during peak bloom. In 20 randomly designated plants, we administered pollen to 4 flowers from the central part of the flowering stalk (PA treatment). Pollen was collected in the early morning from

newly opened flowers of donor individuals located at least 10 m from the receiving individual. In each receiving individual, we selected 4 additional flowers that were left untreated and served as internal controls (IC treatment). IC flowers were located immediately above or below PA flowers. By selecting PA and IC flowers from the central part of the flowering stalks, we expected to avoid potential confounding effects of flower position on reproductive

**Fig. 1** Locations of the 21 *Erysimum popovii* populations studied, and composition of flower visitor functional groups at each site. Numbers are flower visitation rates (in visits  $\text{min}^{-1}$ ); asterisks denote populations in which we could analyze pollen limitation



outcome (Wesselingh 2007). In the 30 remaining plants, we selected 3 flowers as external controls (EC treatment). EC flowers were used to detect possible effects of pollen supplementation on resource allocation (Wesselingh 2007). Domestic and wild ungulates consumed some of our experimental plants. As a result, we could recover enough individuals to perform statistical analyses in only 13 of the 21 populations. For this reason, all subsequent pollen limitation analyses are based on 13 populations.

We measured the effect of pollen limitation during both the pre-dispersal stage of the plant's reproductive success (seed production), and the post-dispersal stage (seedling emergence rate). Seed production was estimated as seeds per flower. At the end of the fruiting season, we collected ripe fruits produced by PA, IC, and EC flowers. For each fruit, ovules, aborted seeds, and ripe seeds were counted in the laboratory using a magnifying glass. We were able to discern ripe seeds, aborted seeds, and unfertilized ovules because, as in other crucifers, ripe seeds are light brown and larger, and aborted seeds are dark brown, with shriveled cotyledons and embryo (Gómez et al. 2010). By contrast, unfertilized ovules are creamy white and smaller. Seedling emergence rate was measured as the proportion of seeds producing seedlings. Due to the relatively low number of seeds obtained in some mother  $\times$  treatment combinations, we decided to pool all seeds within each

treatment and population, and randomly selected 24 seeds per treatment and population (PA, IC, and EC). In February 2010, these seeds were sown in seedbeds in a greenhouse, and emergence was monitored weekly until April.

To determine the magnitude of pollen limitation, we used a pollen limitation (PL) index calculated as  $1 - (RS_C / RS_{PA})$ , where  $RS_C$  is the mean reproductive success of the control treatment and  $RS_{PA}$  the mean reproductive success of the pollen addition treatment. This index has a straightforward biological interpretation (Lázaro and Traveset 2006). Positive values result from higher reproductive success in PA than C, thus indicating pollen limitation, while zero or negative values indicate absence of pollen limitation. We calculated the PL index for both components of reproductive success (seed production and seedling emergence), and for the comparisons PA versus IC and PA versus EC (henceforth  $PL_{IC}$  and  $PL_{EC}$ ). The difference between these two PL estimates provides a measure of resource allocation as a consequence of the experimental supplementation of pollen (Gómez et al. 2010).

#### Flower visitor assemblage

We conducted surveys of floral visitors in each of the 21 populations during the springs of 2008 and 2009. Each population was sampled on several days throughout its

flowering period. Groups of plants with approximately 100 flowers were tagged and repeatedly observed over the course of the day (from 1100 to 1700 hours) and all insects seen actively visiting the flowers (collecting pollen and/or nectar) were noted. We tried to record a minimum of 200 flower–pollinator contacts per population because this number provided a good estimate of overall pollinator assemblage in *E. mediohispanicum* (Gómez et al. 2007). Total survey time per population ranged from 190 to 795 min. We observed a total of 5,169 flower visits (193–331 per population). Specimens ( $n = 486$ ) of most morphospecies were captured for later identification in the laboratory.

We estimated flower visitation rate as the number of flower visits observed per minute of observation. We also quantified the visitation rate of each type of flower visitor, considering the following five functional groups: large bees (10 mm or more in body length), small bees (<10 mm in body length), butterflies (mostly Rhopalocera, all nectar collectors), beeﬂies (long-tongued Bombyliidae), and others (including ants, beetles, hoverﬂies, and other minor groups previously shown to have low pollinating efficiency on *Erysimum*; Gómez et al. 2007, 2008, 2009). Finally, we estimated the overall diversity of the flower visitor community in each population using Hurlbert’s PIE (EcoSim 7; Gotelli and Entsminger 2009). This index ranges between 0 (low diversity) and 1 (maximum diversity), and measures the probability that two randomly sampled individuals from the community belong to different species.

#### Plant population characteristics

We measured the following variables to characterize the *E. popovii* populations: population size (all reproductive individuals in each population), altitude (meters above sea level measured with a GPS), annual rainfall (in mm, obtained from the Digital Climatic Atlas of the Iberian Peninsula; Ninyerola et al. 2005), density of co-occurring flowering plants (flowers per m<sup>2</sup>), woody plant cover (percent area occupied by woody plants), bare soil cover (percent area occupied by bare soil), and rock cover (percent area occupied by rocks). Flowering plant density was measured along three transects of 10 × 2 m in which we counted the number of flowers of species blooming at the same time as *E. popovii*. Habitat cover was estimated along three additional transects of 25 × 2 m in which, every 0.5 m, we scored microhabitat type in the center and at the two edges of the transect width.

#### Data analysis

Significance of the PL index for seed production of each population was obtained with bootstrapping, running 1,000 replicates per population (Boot package; R Development

Core Team 2008). Bootstrapping could not be applied to post-dispersal PL results because, by pooling all seeds within each treatment and population, internal variation required for this analysis was lost (Davison and Hinkley 1997). Thus, we used contingency tables to compare seedling emergence rates across treatments within each population.

Spatial autocorrelation of PL indices was analyzed with Moran’s I coefficient at different distance classes (Rangel et al. 2010). The number of distance classes was established by the default function of the software (SAM v.4.0; Rangel et al. 2010). The relationship of flower–visitor community and population characteristics with pollen limitation intensity was explored by means of a model selection approach. We run a set of models including all possible combinations of independent variables. Since pollen limitation was spatially autocorrelated (Appendix 1), these models were spatially-explicit, including *X* and *Y* geographic coordinates as co-variables to control for the possible effects of spatial distribution. Prior to running these models, we checked for multicollinearity by performing pairwise Pearson’s correlations amongst variables. Since no strong correlation appeared between variables (Appendices 2 and 3), we included all independent variables in the models. Due to the high number of independent variables with respect to the number of samples (13 populations), we ran separate models for flower–visitor and population variables. To further avoid potential problems derived from the small number of populations, we decided not to include more than three independent variables in each model. We thus ran all models resulting from all possible combinations of one, two, or three variables. All analyses were performed with SAM v.4.0 (Rangel et al. 2010). Because the two PL indices were consistent (see below), we only show outcomes of PL<sub>EC</sub>, which is more robust to resource reallocation (Wesselingh 2007).

To select the best model(s), we used an information-theoretic approach (Burnham and Anderson 2002; Richard 2005; Stephens et al. 2007). We first selected those models providing an appropriate goodness of fit ( $P < 0.05$ ; Grace 2006). For this subset of candidate models, we calculated the Akaike information criterion (AIC) and the Akaike weights (AIC<sub>wi</sub>). All models having AIC<sub>wi</sub> > 0.2 were considered an appropriate representation of the raw data (Burnham and Anderson 2002). By means of a multimodel inference process, we determined the importance of each independent variable across all models weighted by their AIC values (Richard 2005).

## Results

### Pollen limitation

Despite large differences among populations, flowers from PA treatments produced more seeds than the two control

treatments in all populations but one (Fig. 2). Excluding population Ep03 (in which IC flowers produced more seeds than PA flowers), PA flowers produced a mean of 3.76 more seeds (SD = 2.03; range 0.74–7.37;  $n = 12$  populations) than IC flowers. When considering entire plants, this represents a mean increase of  $155 \pm 115$  seeds per individual (range 26–416). Of the 13 populations analyzed,  $PL_{IC}$  was significant in 8, and  $PL_{EC}$  in 10 (Table 2), although PL values were mostly moderate ( $PL_{IC} = 0.18\text{--}0.45$ ;  $PL_{EC} = 0.16\text{--}0.49$ ). When pooling all populations, pollen limitation for seed production was significantly different from zero for both PL indices (Table 2).

On the other hand, we found no significant pollen limitation for seedling emergence rate ( $PL_{IC} = 0.012$ ,  $P = 0.46$ ;  $PL_{EC} = -0.039$ ,  $P = 0.31$ ). In fact, only two populations (Ep01 and Ep15) were pollen limited for seedling emergence and only when comparing PA and EC treatments (Table 2).

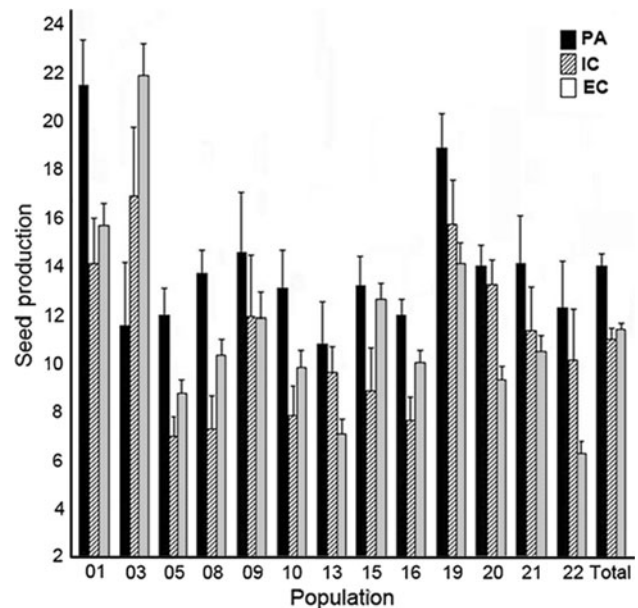
We did not find a consistently better performance of EC treatment compared to IC treatment (binomial test of EC performance > IC performance,  $P = 0.5$ ), with no differences between EC and IC in seed production when data from all populations were lumped together (Fig. 2). In addition, in most populations (9 out of 13),  $PL_{IC}$  and  $PL_{EC}$  values were similar (Table 2). These results provide no evidence of reallocation between internal control and pollen-supplemented flowers.

#### Flower visitors

The flower visitor community of *Erysimum popovii* was very diverse, including large bees, small bees, beesflies, butterflies, hoverflies, beetles, and ants (Fig. 1). Taking all populations together, we observed 166 insect species visiting the flowers of *E. popovii*, ranging from 11 to 32 species per population (Appendix 4). Flower visitor diversity ranged between 0.62 and 0.94 (Hurlbert's PIE index). Mean visitation rate was 0.348 visits per flower per hour (range 0.084–1.050) (Appendix 4). Large bees and beesflies were the most abundant flower visitors (Fig. 1).

#### Factors associated with pollen limitation

As we found no evidence of pollen limitation on germination rates, analyses exploring the factors associated to pollen limitation were only conducted for seed production. The visitation rate of beesflies was the main variable influencing pollen limitation, with an importance value of 0.966, which is much higher than the importance values of the others variables (Table 3). Consequently, the best model included only beesfly visitation rate (Model A; Tables 3, 4). According to this model, beesfly visitation rate was negatively correlated with the intensity of pollen



**Fig. 2** Seed production (seeds per flower) in PA (pollen addition), IC (internal control), and EC (external control) flowers from populations for which we could conduct bootstrapping analysis of pollen limitation (see Table 2). Bars means (+SE)

limitation (Table 3), so that populations with higher beesfly abundance showed less pollen limitation. The abundance of the other flower visitor groups (large bees, small bees, butterflies, and others), as well as the visitation rate and diversity of the whole flower visitor assemblage revealed no relationship to pollen limitation (Table 3). These variables showed very low importance values (Table 3), and did not appear in the selected models (Table 4).

When exploring the effect of population characteristics, we found that annual rainfall and bare soil cover were the two most important variables (0.50 and 0.44 importance values, respectively; Table 3). The selection model process yielded two equally good models (Models B and C; Table 4). Annual rainfall was positively and significantly related to pollen limitation intensity (Table 3). That is, pollen limitation was stronger in populations receiving more precipitation. Model C only included bare soil cover, which was marginally and negatively correlated with  $PL_{EC}$  (Table 3). In other words, pollen limitation tended to be lower in populations with extensive bare soil cover. The remaining population characteristics did not correlate with pollen limitation intensity (Table 3).

#### Discussion

Our study has shown that *E. popovii* reproduction was pollen limited throughout most of its distribution range.

**Table 2** Pollen limitation (PL) intensity in each population

<i>E. popovii</i> population	No. of individuals treated	No. of individuals recovered	Seed production <sup>a</sup>		Seedling emergence rate <sup>b</sup>	
			PL <sub>IC</sub>	PL <sub>EC</sub>	PL <sub>IC</sub>	PL <sub>EC</sub>
01	20	8	0.30 (0.19, 0.5)	0.27 (0.11, 0.45)	0.33 (0.08)	0.40 (0.05)
02	20	3	0.4	0.016	0.15 (0.17)	0.04 (0.38)
03	20	11	−0.89 (−0.89, 0.03)	−0.92 (−1.84, 0.27)	−0.17 (0.39)	−0.50 (0.07)
04	20	5	0.28	0.19	−0.02 (0.71)	0.29 (0.06)
05	20	14	0.36 (0.29, 0.54)	0.27 (0.12, 0.43)	−1 (0.11)	0.60 (0.16)
07	20	2	−0.25	0.14	0 (0.99)	−1 (0.28)
08	20	7	0.45 (0.27, 0.67)	0.24 (0.11, 0.38)	−	0 (0.99)
09	20	6	0.14 (−0.07, 0.45)	0.18 (−0.07, 0.45)	0 (0.99)	−1 (0.01)
10	20	7	0.39 (0.3, 0.5)	0.25 (0.29, 0.5)	0 (0.05)	−8 (0.005)
13	20	16	−0.15 (−0.02, 0.25)	0.34 (0.08, 0.65)	−	−
14	20	5	0.12	−0.41	0.18 (0.1)	0.04 (0.16)
15	20	11	0.25 (0.05, 0.62)	0.043 (−0.17, 0.27)	0.20 (0.19)	0.60 (0.04)
16	20	12	0.35 (0.21, 0.5)	0.16 (0.03, 0.31)	−0.62 (0.12)	−0.87 (0.06)
19	20	17	0.18 (0.04, 0.29)	0.25 (0.1, 0.4)	0 (0.25)	−1 (0.04)
20	20	16	0.016 (−0.09, 0.19)	0.33 (0.24, 0.43)	−0.05 (0.5)	−0.05 (0.5)
21	20	14	0.22 (0.07, 0.31)	0.26 (0.05, 0.48)	0 (0.33)	0.10 (0.23)
22	20	8	0.17 (−0.01, 0.36)	0.49 (0.34, 0.67)	−1.17 (0.04)	0 (0.26)
23	20	3	0.36	0.21	−	0.50 (0.11)
27	20	5	0.26	0.24	0 (0.28)	0.11 (0.21)
28	20	5	0.12	0.01	0.75 (0.15)	−2 (0.01)
30	20	0	−	−	−	−
Total	420	175	0.21 (0.16, 0.27)	0.19 (0.12, 0.25)	0.012 (0.46)	−0.039 (0.31)

IC internal control, EC external control, Total the number of individuals from all populations

<sup>a</sup> The significance of PL for seed production was determined through bootstrapping with 1,000 permutations (only in those populations in which more than eight individuals could be recovered). Populations in which the bootstrapping 95 % confidence interval (in parentheses) does not include zero have significant pollen limitation

<sup>b</sup> For seedling emergence rate, the significance of PL was tested with Fisher's exact test (*P* values in parentheses)

This finding was somewhat unexpected because *E. popovii* is self-compatible and its flowers are visited by over 166 insect species belonging to very disparate functional and taxonomic groups, from large bees and butterflies to hoverflies, beesflies, beetles, and ants. In addition, intrapopulation diversity of flower visitors was very high, with Hurlbert's PIE values around 0.84. These results indicate that the pollination system of *E. popovii* is very generalist, as found in other *Erysimum* species, such as *E. mediohispanicum*, *E. nevadense*, and *E. baeticum* (Gómez et al. 2007; Ortigosa and Gómez 2010). Self-compatible and/or pollinator generalist plants are less prone to pollen limitation than self-incompatible and/or specialist species (Ashman et al. 2004; Knight et al. 2005). Nevertheless, pollen limitation has been found in another *Erysimum* species with a similar pollination system (Gómez et al. 2010). In *E. popovii*, pollen limitation affected seed production rather than emergence rate. This outcome points to a quantitative rather than a qualitative pollen limitation

(Aizen and Harder 2007). However, because inbreeding depression in self-compatible species is more pronounced in late stages of the life cycle (Husband and Schemske 1996), further studies would be needed to confirm this conclusion in *E. popovii*.

Resource reallocation from untreated to pollen-supplemented flowers is always a concern in PL experiments. To avoid confounding results related to resource reallocation, some studies have submitted whole individuals to control or experimental treatments (Baker et al. 2000). Although we could not follow this approach, because *E. popovii* individuals often produce several hundred flowers, we used two complementary controls, one internal and the other external, to detect potential reallocation (Wesselingh 2007; Gómez et al. 2010). We found that the reproductive output of the two control treatments was similar and consistent, suggesting that resource reallocation, if existing, was weak. In fact, under severe resource reallocation, we would have consistently expected better performance by external rather

**Table 3** Variables influencing pollen limitation intensity for seed production, based on 13 populations in which bootstrapping analysis could be conducted

	Multi-model inference Importance	Statistical outcomes of best models			
		Coeff. $\pm$ SE	<i>t</i>	<i>P</i> value	Model
Flower visitor variables					
Beefly visitation rate	0.966	-1.604 $\pm$ 0.369	-4.347	0.002	A
Small bee visitation rate	0.046				
Large bee visitation rate	0.042				
Flower visitor diversity (Hurlbert's PIE)	0.041				
Flower visitation rate (insects min <sup>-1</sup> )	0.039				
Other pollinators visitation rate	0.039				
Butterfly visitation rate	0.037				
Population variables					
Annual rainfall (mm)	0.504	0.010 $\pm$ 0.004	2.34	0.047	B <sup>a</sup>
Bare soil cover (%)	0.44	-5.56 $\pm$ 2.43	-2.28	0.052	C
Rock cover (%)	0.108				
Co-occurring flower density (plants m <sup>-2</sup> )	0.09				
Population size (N° of <i>E. popovii</i> plants)	0.077				
Woody plant cover (%)	0.044				
Altitude (m a.s.l.)	0.042				

For each variable, the importance value obtained from a multi-model inference using AIC weights averaged across 127 models. Mean  $\pm$  SE coefficients were obtained from the model-averaging process

The statistical outcomes of the best models are given in Table 4

<sup>a</sup> To obtain meaningful slopes in Model B, rainfall was measured in m instead of mm

than internal control flowers. However, this was not so, as only half the populations showed this pattern. In addition, we found a high degree of congruence between our two measures of pollen limitation (PL<sub>IC</sub> and PL<sub>EC</sub>), which showed the same trend in 12 of the 13 populations that could be statistically analyzed (Table 2). Finally, we found no differences between the two controls for seedling emergence rate.

Variation among populations in pollinator assemblage may produce inter-population differences in pollen movement patterns and pollen transfer effectiveness, because different types of flower visitors have different flower-handling behaviors and show important differences in the amount and quality of pollen deposited per visit (Gómez and Zamora 1999; Fenster and Dudash 2001; Cosacov et al. 2008). Consequently, several studies have found that plant populations differ in the intensity of pollen limitation as a consequence of differences in pollinator abundance and diversity (Baker et al. 2000; Gonzalez-Varo et al. 2009; Gómez et al. 2010). In *E. mediohispanicum*, a co-occurring and ecologically similar species to *E. popovii*, pollinator diversity and abundance significantly affect pollen limitation (Gómez et al. 2010). Under this perspective, it is remarkable that none of the two variables were correlated with pollen limitation intensity in *E. popovii*. *E. mediohispanicum* was found to be pollen limited in 50 % of the populations studied (Gómez et al. 2010), whereas *E. popovii* is pollen limited throughout most of its geographic range. We believe that this disparity between the two plant species is a consequence of differences pollinator visitation rate between both species. Pollinator visitation rates in

*E. popovii* (mean  $\pm$  SD: 0.348 visits flower<sup>-1</sup> h<sup>-1</sup>; Appendix 4) are three times lower than those reported for *E. mediohispanicum* (mean  $\pm$  SD: 0.960 visits flower<sup>-1</sup> h<sup>-1</sup>; Gómez et al. 2007). More important, some populations received an even lower amount of visits. Since *E. popovii* flowers last only 1 or 2 days, we believe that this difference in pollinator abundance at flowers may cause the strong pollen limitation observed in this plant species. These differences could be due to traits intrinsic to each species (e.g., *E. popovii* individuals produce fewer flowers than *E. mediohispanicum*; Blanca et al. 2009), or to extrinsic factors such as differences in co-blooming plant composition. Lay et al. (2011) have recently found *Erysimum capitatum* not to be pollen limited, despite having a pollinator visitation frequency similar to *E. mediohispanicum*. They argue that this could be due to *E. capitatum* pollinators being more effective than those of *E. mediohispanicum*.

In *E. popovii*, beefly abundance was associated with low intensity of pollen limitation. Beeflies are likely to be very efficient pollinators of *E. popovii* because, compared to most other flower visitors, they move frequently between plants and visit few flowers per individual. This kind of foraging behavior has also been observed on *E. mediohispanicum* (Gómez et al. 2011). This foraging pattern potentially maximizes deposition of allogamous pollen per visit, which may reduce abortion rate and decrease pollen limitation during pre-dispersal stages (Vaughton and Ramsey 2010). Beeflies have been shown to be highly efficient pollinators in other plant species (Motten et al. 1981; Johnson and Dafni 1998; Kastinger and Weber 2001;



**Table 4** Outcome of the model selection process for flower visitor and population variables

Model	Variables	AIC <sub>wi</sub>
Flower visitor variables		
Model A	3	0.797
Model 2	3, 7	0.036
Model 3	3, 4	0.035
Model 4	3, 6	0.028
Model 5	1, 3	0.025
Model 6	3, 5	0.022
Model 7	2, 3	0.02
Population variables		
Model B	10	0.312
Model C	13	0.277
Model 3	10, 13	0.075
Model 4	14	0.046
Model 5	11	0.041
Model 6	10, 14	0.037
Model 7	11, 13	0.024
Model 8	8, 13	0.022
Model 9	8, 10	0.02

Only models having an AIC weight (AIC<sub>wi</sub>)  $\geq$  0.02 are shown

Variables: 1 flower visitation rate, 2 flower visitor diversity, 3 bee fly visitation rate, 4 butterfly visitation rate, 5 large bee visitation rate, 6 small bee visitation rate, 7 others visitation rate, 8 population size, 9 altitude, 10 annual rainfall, 11 co-occurring flowering plants, 12 woody plant cover (%), 13 bare soil cover (%), 14 rock cover (%)

Anderson et al. 2005; Koopman and Ayers 2005), including the con-specific *E. mediodispanicum* (Gómez et al. 2009, 2011). These results show that the abundance of highly efficient pollinator groups, rather than the abundance of the overall flower visitor assemblage, may be the most important determinant of pollen limitation in some pollination systems (Lay et al. 2011).

Our experiments also demonstrate that some environmental variables, such as annual rainfall and bare soil cover, are associated with pollen limitation in *E. popovii*. Annual rainfall was positively related to pollen limitation. This is probably due to the fact that plants growing in areas with low rainfall are unable to respond to supplementary pollination by increasing their seed number. In other words, in populations with low rainfall, seed production is likely to be limited by water availability or by a combination of water availability and pollen (Haig and Westoby 1988). Bare soil cover had a marginally significant negative effect on pollen limitation. A potential explanation could be related to the fact that most bees nest underground in areas deprived of vegetation. Consequently, bare soil availability may enhance the abundance and diversity of bees and other ground-nesting pollinators (Potts et al. 2005). In addition, by promoting bee abundance, bare soil

may also enhance bee fly abundance, as most bee flies are parasitic on ground-nesting bees and wasps (Boesi et al. 2009).

In sum, our study shows that *E. popovii* is pollen limited throughout most of its geographic range, and, although visited by a diverse community of pollinating insects, the intensity of pollen limitation in this species is mostly attributable to a specific pollinator group, namely bee flies.

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