



GfÖ

GfÖ Ecological Society of Germany,  
Austria and Switzerland

Basic and Applied Ecology xxx (2015) xxx–xxx

Basic and  
Applied Ecology

www.elsevier.com/locate/baae

## Variation in the reproductive success of a narrow endemic plant: Effects of geographical distribution, abiotic conditions and pollinator community composition

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Received 2 June 2014; received in revised form 16 February 2015; accepted 20 February 2015

### Abstract

Geographic variation in reproductive output determines plant distribution. In this study, we investigate the geographic structure and the factors affecting reproductive success throughout the life cycle of the near-threatened crucifer *Erysimum popovii* across its entire distribution range. We worked in 21 populations, in which we measured fruit set, seed set, seed weight, seed germination in the laboratory, germination time, seedling emergence in the field, seedling survival and fecundity. We also sampled the pollinator assemblages visiting *E. popovii* at each site, as well as some population characteristics (population size and density, flower density of *E. popovii* and other co-occurring species, and rainfall). Germination success in the laboratory was very high (range: 0.56–0.98), but seedling emergence in the field was low (0.005–0.32). Bee fly visitation rate was positively related to seedling emergence, whereas visitation rate by ants, beetles and other minor pollinator groups was negatively related to fruit set and positively related to germination time. Populations in sites with high density of co-occurring flowers produced fewer fruits. Most variables related to reproductive output varied widely across populations, but this variation did not show a clear regional structure. The low seedling survival may constitute a bottleneck for the recruitment of this species. Overall, less than 0.2% of the ovules produced developed into reproductive individuals. Our results suggest a metapopulation structure for *E. popovii*.

### Zusammenfassung

Geographische Variabilität beim Reproduktionsergebnis bestimmt die Verteilung von Pflanzen. Hier untersuchen wir die geographische Struktur und die den Reproduktionserfolg bestimmenden Faktoren im Lebenszyklus des potentiell gefährdeten Kreuzblüters *Erysimum popovii* in seinem gesamten Verbreitungsgebiet. Wir untersuchten 21 Populationen und maßen Fruchtansatz, Samenansatz, Samengewicht, Keimung im Labor, Keimungszeit, Auflaufen der Keimlinge im Freiland, Überleben der Keimlinge und Fekundität. Wir erfassten auch die Bestäubergemeinschaften von *Erysimum popovii* an jedem Standort sowie einige Populationsparameter (Populationsgröße und -dichte, Blütendichte von *Erysimum popovii* und vergesellschafteten Arten und den Niederschlag). Der Keimungserfolg im Labor war sehr hoch (0.56 bis 0.98) aber im Freiland erschienen nur wenige Keimlinge (0.005 bis 0.32). Die Besuchshäufigkeit durch Wollschweber war positiv mit dem Keimungserfolg verbunden,

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<http://dx.doi.org/10.1016/j.baae.2015.02.006>

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während Besuche von Ameisen, Käfern und anderen weniger bedeutenden Bestäubergruppen negativ mit dem Fruchtansatz und positiv mit der Keimungszeit verbunden waren. Populationen an Standorten mit einer hohen Dichte von vergesellschafteten Blüten produzierten weniger Früchte. Die meisten den Reproduktionserfolg kennzeichnenden Variablen variierten stark von Population zu Population, aber diese Variation zeigte kein klares regionales Muster. Die geringe Überlebensrate der Keimlinge könnte einen Flaschenhals für die Rekrutierung von Nachkommen bei dieser Art darstellen. Insgesamt entwickelten sich weniger als 0.2% der Samenanlagen in ein reproduktives Individuum. Unsere Ergebnisse legen für *Erysimum popovii* eine Metapopulationsstruktur nahe.

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**Keywords:** *Erysimum popovii*; Fitness; Fruit set; Germination; Germination time; Reproduction; Seed set; Seedling; Seed weight

## Introduction

The study of factors determining plant reproduction is crucial to understand species distribution. Most studies addressing this issue focus on demographic analyses, and include various phases of the plant's life cycle such as fruit set, seed production and seed germination (e.g. Menges 1991; Colas, Olivieri, & Riba 2001; Rebollo, Perez-Camacho, Garcia-de, Benayas, & Gomez-Sal 2001; Metz et al. 2010). Overall, limitation of reproduction in pre-dispersal phases (fruit set and seed production) may be determined by different factors such as the genetic constitution of the mother plant, pollen limitation (Knight et al. 2005; Aizen & Harder 2007; Fernández, Bosch, Nieto-Ariza, & Gómez 2012), ovule abortion (Stephenson 1981), resource limitation (Bloom, Chapin, & Mooney 1985), and predation (Grieg 1993). Fruit set has often been found to be low in many plants (Ayre & Whelan 1989), supporting the view that flowers represent a reservoir of ovules that can be used when resources are plentiful, thus providing an adaptive response to stochastic environments. Instead, under sub-optimal conditions, plants may save resources by discarding flowers or fruits at the beginning of their development (Ayre & Whelan 1989). In addition to fruit set, final seed yield, may also be limited by low seed set within fruits (ratio of ripe seeds to ovules). Limitation of reproduction in the post-dispersal phase (seed germination) has commonly been linked to plant population size (Menges 1991), density of conspecific and heterospecific plants, abiotic conditions such as precipitation regime (Venable 2007), and seed quality (Winn 1985; Burke & Grime 1996). Two of the most widely used estimators of seed quality are seed weight and germination time. Seed weight affects many processes of the reproductive cycle, including seed germination (Naylor 1993), dormancy period (Stamp 1990; Fenner & Thompson 2004), resistance to competition (Houssard & Escarré 1991; Turnbull, Coomes, Hector, & Rees 2004), seed dispersal (Fenner & Thompson 2004), and seedling growth and recruitment (Coomes & Grubb 2003). Germination time, on the other hand, may be especially determinant in annual and herbaceous plant species at the community level via competitive dominance of the early germinating individuals. Very short delays in emergence time can be amplified in the long

term, with strong effects on final biomass and reproductive output (Weiner & Thomas 1986).

Therefore, different life cycle phases are affected by different extrinsic biotic and abiotic factors, which in addition, may vary spatially at different scales (Anderson & Johnson 2008; Nattero, Sérsic, & Cocucci 2011). That is, the relative contribution of the different phases of the life cycle to the plant's reproductive output may vary spatially depending on the locally prevailing biotic interactions and abiotic conditions (Stevens, Bunker, Schnitzer, & Carson 2004; Kim & Donohue 2011). Pollinator abundance, composition and diversity have often been related to plant reproductive success (Steffan-Dewenter et al. 2001; Cosacov, Naretto, & Cocucci 2008; González-Varo, Arroyo, & Aparicio 2009; Gómez, Abdelaziz, Lorite, Muñoz-Pajares, & Perfectti 2010). Pollen limitation is often associated with low pollinator abundance (Cosacov et al. 2008; González-Varo et al. 2009; Gómez et al. 2010), and different pollinator species usually differ in pollinating effectiveness and may thus have different effects on plant fitness (Klein, Steffan-Dewenter, Buchori, & Tscharntke 2002; Gómez, Bosch, Perfectti, Fernández, & Abdelaziz 2007; Perfectti, Gómez, & Bosch 2009). Additionally, some habitat characteristics such as plant population size, habitat fragmentation, co-occurring flower composition, and altitude can influence plant reproduction either directly or indirectly via their effect on biotic interactions (González-Varo et al. 2009; Kim & Donohue 2011). Understanding how these biotic and abiotic variables affect different phases of the reproductive cycle is essential to interpret the reproduction dynamics, geographical structure and conservation status of a plant species (Colas et al. 2001; Metz et al. 2010).

In this study, we explore the geographic variation of reproductive success and recruitment, as well as their relationship with local pollinator assemblages and abiotic conditions in *Erysimum popovii*, a narrow endemic Brassicaceae species. Our objectives are: (1) to study the different phases of the reproductive cycle and evaluate their relative contribution to recruitment; (2) to analyze the geographic structure of the reproductive output across the entire distribution range of the species; (3) to measure the effect of pollinator diversity, abundance and composition on the various phases of the reproductive cycle; and (4) to measure the effect of *E.*

*popovii* population characteristics and habitat composition on the various phases of the reproductive cycle.

## Materials and methods

### Study system

*E. popovii* Rothm. (Brassicaceae) is a narrow endemic species from southeastern Spain, categorized as “Near Threatened” in the Red List of Andalusian Vascular Flora (Cabezudo et al. 2005). The species shows a scattered distribution across the Baetic mountains from 900 to 2000 m a.s.l., inhabiting rocky areas and shrubland gaps. *E. popovii* is a biennial to perennial herb that, under natural conditions, requires at least 2 years to complete its reproductive cycle. However, under optimal conditions (such as in a greenhouse), plants may produce seeds in the first year. Flowering occurs between May and June, depending on the altitude.

Each plant produces from a few to several hundred flowers on a variable number of reproductive stalks. Flowers are hermaphroditic, slightly protandrous, with four lilac petals and a tetradynamous androecium (four long and two short stamens) (Blanca et al. 2009). Seeds ripen in late September, and seedlings emerge in winter. Like other *Erysimum* species (Gómez 2005b), *E. popovii* is partially self-compatible and requires pollen vectors for full seed set (unpublished data).

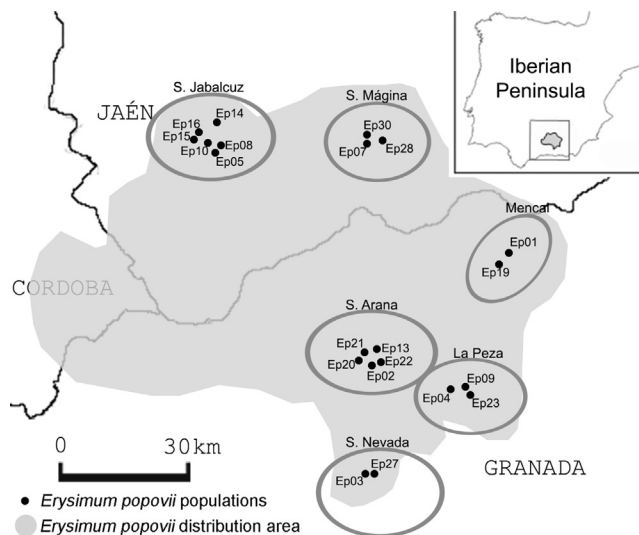
The study was conducted in 21 *E. popovii* populations located in the Granada and Jaén provinces, spanning the entire geographic range of the species (see Appendix A: Fig. 1). These populations are distributed across 6 regions (Mencal, Sierra Arana, Sierra Nevada, La Peza, Sierra de Jabalcuz, Sierra Mágina) (Fig. 1). After fieldwork had been completed, two populations from the Mencal region (Ep01 and Ep19, see Appendix A) were provisionally reassigned

to the related taxon *Erysimum bastetanum*. However, and because taxonomic relationships are far from being resolved in *Erysimum* species from southeastern Spain, we decided to keep these two populations in our study. Geological substrates are mainly limestone and dolomites (Vera 2004). The overall area has a characteristic Mediterranean climate type, with cool wet winters and warm dry summers. The annual average rainfall ranges between ca. 500 and 1000 mm, and annual mean temperatures between 9.2 and 14.2 °C.

### Reproductive output

We measured several variables related to reproductive success:

- (1) *Fruit set*. The proportion of flowers that produced fruits, estimated in the field in September of 2008 and 2009. In 30 randomly selected plants per population, we collected all fruits produced and counted all indehiscent pedicels (a direct measure of the number of flowers produced the previous year).
- (2) *SO ratio*. Seed to ovule ratio, measured as the number of ripe seeds per ovule. Four ripe fruits in each of the 30 above-mentioned plants were taken to the laboratory and the number of seeds was counted under a magnifying lens. We were able to distinguish aborted seeds from unfertilized ovules because, as in many other crucifers, *Erysimum* aborted seeds are dark brown, with shriveled cotyledons and embryo (Gómez et al. 2010), whereas virgin ovules are creamy white, lanceolate in shape and of much smaller size.
- (3) *Seed weight (in mg)*. For each population, we pooled together the seeds produced by the 30 above-mentioned individuals. Then, we weighed three samples of ten randomly chosen seeds per population and calculated the average of the three weights. Reported values express weight per 100 seeds in each population.
- (4) *Seed germination*. We recorded the germination of 125 randomly selected ripe seeds per population under laboratory conditions. Seeds were distributed in 5 pasteurized 90-mm Petri dishes (25 seeds per dish) containing a layer of small glass balls (ca. 3 mm diameter) with a filter paper disk on top. Afterwards, we added 10 ml of distilled water. To avoid mold propagation, all seeds were previously disinfected with 5% sodium hypochlorite solution for 3 min. Dishes were placed in a germination chamber, with a day/night cycle of 16 h/8 h at 16 °C/24 °C ± 2 °C. We chose these conditions to simulate field conditions during autumn. Seeds that produced a radicle protruding 1 mm or more were scored as germinating. Germination checks were conducted three times per week, during 60 days. After each check, dishes were randomly shuffled within the chamber to avoid potential position effects.
- (5) *Germination time*. The time needed to reach 50% of total germination (T50, in days) (Kos & Poschlod 2010). We averaged the germination times in each of the five



**Fig. 1.** Geographical distribution of the 21 *E. popovii* populations studied grouped by region.

above-mentioned Petri dishes. Low T50 values denote high germinating speeds.

- (6) *Seedling emergence*. This parameter was determined experimentally under field conditions. At the end of winter 2009, we planted 200 seeds per population. These seeds were again randomly chosen from the groups of seeds described above. We distributed the 200 seeds in ten 30 cm × 15 cm replicates of 20 seeds each. Within each replicate, seeds were placed regularly, with a distance of 4 cm between seeds to avoid competition effects during the germination process. Replicates were haphazardly distributed across the entire area occupied by the population irrespective of microhabitat. Once a month we counted the number of emerged seedlings.
- (7) *Seedling survival*. This parameter was also determined under field conditions. In November of 2009 we conducted a seed supplementation experiment. Seeds were sown in 0.25 m<sup>2</sup> permanent quadrats, replicated 10 times across each population. Seeds came from a common pool from all plants within each population. The number of seeds used per replicate was 10 times the estimated seed density of each population (obtained from data on population size, fruit set and SO ratio in each population) (see Appendix B). We used such a high seed density to ensure a sufficient number of juveniles in spite of the low rate of seedling emergence, and to account for the aggregated seed distribution observed in natural populations of *E. popovii*. In each population, we set up 10 randomly located replicates including the different microhabitats. From December 2010 to May 2011 we counted the juveniles (pre-adult rosettes) growing in the quadrats.
- (8) *Fecundity*. We quantified fecundity as the mean production of juveniles per reproductive adult, and used this quantity as an inclusive estimate of the plant reproductive output in each population. The transition from juvenile to adult is near 1, as almost all juveniles become adults (field and greenhouse observations).

### Pollinator assemblages

In the springs of 2008 and 2009 we recorded the identity and abundance of pollinators visiting the flowers of *E. popovii* in each population. Surveys were carried out from 11:00 am to 17:00 pm (GMT +2) on several days throughout the flowering period, under suitable weather conditions (sunny days with little or no wind and temperatures higher than 18 °C). Observations were done on groups of plants comprising about 100 flowers. Total survey time per population ranged between 190 and 795 min, depending on pollinator abundance. We tried to reach a minimum of 200 pollinators per population, a sampling effort adequate to describe pollinator assemblages in other *Erysimum* species (Gómez et al. 2007; Gómez, Muñoz-Pajares, Abdelaziz, Lorite, & Perfectti 2014). We only recorded those insects contacting the sexual parts of the flower, thus acting as legitimate pollinators. We

observed a total of 5169 insects, ranging from 193 to 331 per population. Specimens ( $n = 486$ ) of most morphospecies were captured for later identification in the laboratory.

We estimated pollinator diversity using the Hurlbert's PIE index, which measures the probability that two randomly sampled individuals from the community belong to different species. This index was computed using EcoSim 7 (Gotelli & Entsminger 2009). In addition, we quantified the visitation rate, measured as the number of visits per hour of survey, of four pollinator functional groups (following Gómez et al. 2007): (1) Large bees: >10 mm in body length; (2) Small bees: <10 mm in body length; (3) Beeflies: long-tongued Bombyliidae; (4) Low quality pollinators: including ants, beetles, hoverflies and other minor groups previously shown to have low pollinating efficiency on *Erysimum* (Fernández & Gómez 2012).

### Plant population characteristics

We characterized each *E. popovii* population with the following variables: (1) Population size: number of reproductive individuals; (2) Population density: number of individuals per square meter; (3) *E. popovii* flowers: estimated number of *E. popovii* flowers per population, based on 30 randomly selected plants per population; (4) Co-occurring flower density, measured along three transects of 10 m × 2 m in each population, in which we counted the number of flowers of all species blooming at the same time as *E. popovii*; and (5) Mean annual rainfall (in mm), obtained from the Digital Climatic Atlas of the Iberian Peninsula (Ninyerola, Pons, & Roure 2005).

### Data analysis

We used Markov chain Monte Carlo multivariate generalized linear mixed models to test for differences among populations and regions in plant reproductive output (MCM-Cglmm package, R Development Core Team 2008). We tested each variable related to reproductive output separately. Fruit set, SO ratio, germination and seedling emergence and survival were fitted to binomial distributions, whereas germination time and seed weight were fitted to Gaussian distributions. We built three models for each reproductive output variable: a first model including only region as fixed factor, a second model including only population nested into region as random factor, and a third model including both independent variables: population nested into region as random factor and region as fixed factor. Then, we compared the three models using the deviance information criterion (DIC), and selected the model with lowest DIC value (Hadfield 2010). DIC is a Bayesian analog of AIC, with a similar justification but wider applicability (Spiegelhalter, Best, Carlin, & Van der Linde 2002). Each model was iterated 50,000 times.

We tested for spatial structure in our dataset. We explored the spatial autocorrelation of the variables with Moran's

*I* coefficient. This coefficient measures whether the values of close populations are more similar than expected by chance (Rangel, Diniz-Filho, & Bini 2010). The number of distance classes was established by the default function of the software SAM v. 4.0 (Rangel et al. 2010) and the significance test was based on 200 permutations.

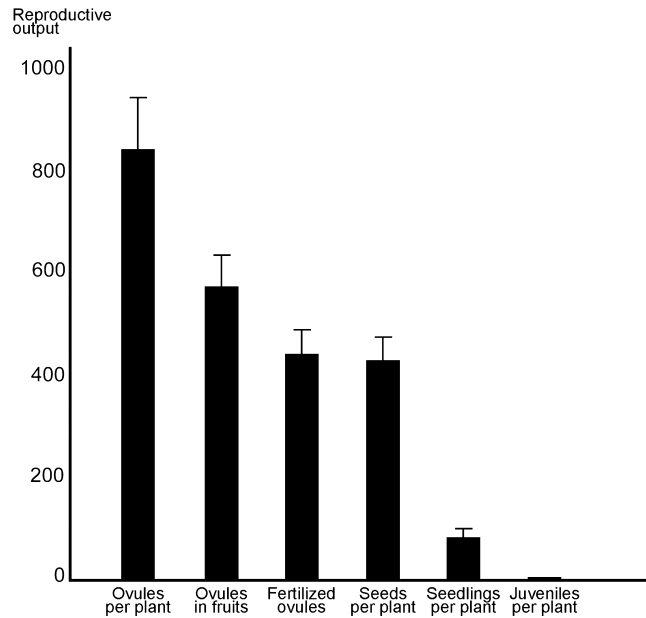
The relationship between *E. popovii* reproductive output, pollinator assemblage, and population characteristics was explored with a set of models including all possible combinations of independent variables. Since some independent and dependent variables were spatially autocorrelated (see Appendices C and D), all models were spatially explicit. Prior to running these models, we checked for multicollinearity by performing pairwise Pearson correlations amongst variables. Since no strong correlation appeared between variables (see Appendices E–G), we included all independent variables in the models. We ran two regression models for each reproductive output estimate, one including five pollinator variables (pollinator diversity, and visitation rate of the four above-mentioned pollinator functional groups), and the other including five population variables (population size, population density, abundance of *E. popovii* flowers, density of flowers of co-occurring species, and annual rainfall). All these analyses were performed with SAM v. 4.0 (Rangel et al. 2010).

## Results

### Reproductive output

Most reproductive output variables varied widely among populations (see Appendix H: Table 1). Number of flowers per individual ranged between 15.2 and 79.5 (mean  $\pm$  SD:  $37.6 \pm 17.9$ ) (see Appendix H). In all populations, more than 50% of the flowers produced fruits (fruit set range: 0.50–0.74, mean  $\pm$  SD:  $0.67 \pm 0.14$ ) (Table 1). The number of ovules per flower ranged between 12 and 39 (see Appendix H). Of these, 2.5% aborted, 23.5% were not fertilized, and the rest (74% SO ratio) produced seeds. SO ratio ranged between 61 and 83% (Table 1). Seed weight per 100 seeds ranged between 0.29 and 0.73 mg (mean  $\pm$  SD:  $0.5 \pm 0.1$ ) (Table 1).

Germination success in the laboratory was very high (mean  $\pm$  SD:  $0.81 \pm 0.15$ ), but again varied widely across populations (range: 0.56–0.98) (Table 1). Germination time (T50), was approximately 5–6 days in all populations except Ep13 (9 days, Table 1). Seedling emergence in the field was much lower, ranging between 0% and 34% (mean  $\pm$  SD:  $14.7\% \pm 14.0\%$ ) (Table 1), so that seedling production per individual ranged between 5 and 240 (mean  $\pm$  SD:  $68.2 \pm 72.2$ ) (see Appendix H). In addition, seedling survival was low (mean  $\pm$  SD:  $0.06 \pm 0.02$ , range: 0–0.34), so that overall fecundity (number of surviving juveniles per reproductive adult was as low as  $1.7 \pm 0.6$ ) (Table 1). Importantly, fecundity ranged from 0 (no recruitment) to 9.4 (Table 1). On average, only 1.7 of the 839 ovules produced per



**Fig. 2.** Reproductive output (mean  $\pm$  SE per individual plant) throughout the reproductive cycle of *E. popovii*.

individual plant resulted in a juvenile with chances of reproducing (Fig. 2).

There were some significant correlations between reproductive output variables. Fruit set was negatively correlated with germination time in the laboratory ( $r = -0.663$ ,  $p = 0.003$ ), and SO ratio was negatively correlated with seed weight ( $r = -0.513$ ,  $p = 0.029$ ) (see Appendix G). Seedling emergence was correlated positively with number of seedlings per plant ( $r = 0.566$ ,  $p = 0.014$ ), and negatively with germination time in the laboratory ( $r = -0.470$ ,  $p = 0.049$ ) (see Appendix G). Differences across populations were significant for all reproductive variables, but we found no differences among regions (Table 2). Four of the eight reproductive variables measured showed spatial autocorrelation at one scale or another (see Appendix C).

### Effect of pollinators

We recorded 166 insect species visiting the flowers of *E. popovii* in the 21 populations. This assemblage was taxonomically diverse, composed of insects belonging to different functional groups, although large bees and bumblebees were the most frequent pollinators, representing 25.9% and 27.6% of the total flower visits, respectively. Pollinator composition varied widely across populations. The number of species recorded per population ranged between 11 and 32, and their diversity (Hurlbert's PIE index), between 0.62 and 0.94 (see Appendix I). Large bee visitation ranged between 0.8 and 44.7 visits/h, and bumblebee visitation between 0 and 48.2 visits/h.

The abundance of low-quality pollinators was negatively related to fruit set and positively related to germination time (Table 3). That is, populations with more low-quality

**Table 1.** Transition probabilities of the reproductive output of the 21 *E. popovii* study populations.

Population	Fruit set	SO ratio	Germination success	Seedling emergence	Seedling survival	Seed weight (mg/100seeds)	Germination time (T50)	Fecundity
Ep01	0.671 ± 0.11	0.781 ± 0.13	0.824 ± 0.16	0.170 ± 0.22	0	0.333 ± 0.042	4.904 ± 0.032	0.00
Ep02	0.642 ± 0.14	0.759 ± 0.11	–	0.050 ± 0.06	–	–	–	–
Ep03	0.711 ± 0.08	0.800 ± 0.18	0.840 ± 0.07	0.340 ± 0.16	0.011	0.400 ± 0.02	4.920 ± 0.07	1.69
Ep04	0.702 ± 0.12	0.831 ± 0.09	0.896 ± 0.12	0.315 ± 0.13	0.156	0.403 ± 0.02	4.819 ± 0.01	9.38
Ep05	0.692 ± 0.11	0.783 ± 0.20	0.779 ± 0.16	0.105 ± 0.06	0.007	0.573 ± 0.09	5.744 ± 0.40	0.27
Ep07	0.661 ± 0.16	0.625 ± 0.18	0.928 ± 0.07	0.100 ± 0.1	0.043	0.733 ± 0.07	4.850 ± 0.02	0.23
Ep08	0.649 ± 0.14	0.815 ± 0.09	0.576 ± 0.19	0.045 ± 0.05	0.052	0.387 ± 0.015	5.962 ± 0.39	0.71
Ep09	0.723 ± 0.27	0.808 ± 0.07	0.815 ± 0.09	0.045 ± 0.05	0.174	0.481 ± 0.07	4.950 ± 0.04	2.22
Ep10	0.684 ± 0.09	0.684 ± 0.16	0.824 ± 0.10	0.105 ± 0.14	0.028	0.637 ± 0.10	5.161 ± 0.08	1.33
Ep13	0.500 ± 0.12	0.725 ± 0.19	0.838 ± 0.08	–	0.338	0.563 ± 0.08	9.303 ± 0.41	–
Ep14	0.720 ± 0.11	0.692 ± 0.14	0.960 ± 0.05	0.225 ± 0.10	0.007	0.547 ± 0.15	5.931 ± 0.88	1.78
Ep15	0.721 ± 0.07	0.819 ± 0.10	0.564 ± 0.15	0.130 ± 0.10	0.009	0.487 ± 0.18	5.261 ± 0.15	1.24
Ep16	0.687 ± 0.10	0.697 ± 0.17	0.687 ± 0.08	0.260 ± 0.13	0.042	0.369 ± 0.06	5.453 ± 0.42	6.39
Ep19	0.671 ± 0.13	0.701 ± 0.13	0.824 ± 0.08	0.105 ± 0.10	0.004	0.360 ± 0.05	5.449 ± 0.13	0.13
Ep20	0.739 ± 0.09	0.676 ± 0.16	0.904 ± 0.09	0.206 ± 0.13	0.037	0.623 ± 0.11	5.614 ± 0.44	2.49
Ep21	0.658 ± 0.10	0.766 ± 0.13	0.976 ± 0.04	0.115 ± 0.10	0.023	0.523 ± 0.01	4.892 ± 0.05	1.02
Ep22	0.550 ± 0.19	0.607 ± 0.18	–	0.110 ± 0.09	–	–	–	–
Ep23	0.632 ± 0.12	0.786 ± 0.10	0.666 ± 0.12	0.175 ± 0.12	0.005	0.347 ± 0.03	5.123 ± 0.17	0.24
Ep27	0.635 ± 0.09	0.731 ± 0.13	0.792 ± 0.16	0.255 ± 0.14	0.002	0.290 ± 0.06	5.033 ± 0.09	0.13
Ep28	0.742 ± 0.09	0.767 ± 0.20	0.792 ± 0.10	0.089 ± 0.07	0.018	0.520 ± 0.04	5.911 ± 0.22	0.46
Ep30	0.695 ± 0.13	–	0.952 ± 0.02	0.155 ± 0.11	0.030	0.537 ± 0.06	4.851 ± 0.03	0.60
Total (mean 21 population)	0.67 ± 0.14	0.74 ± 0.16	0.81 ± 0.15	0.15 ± 0.14	0.058 ± 0.02	0.480 ± 0.13	5.481 ± 0.23	1.68 ± 0.57

**Table 2.** DIC values of models relating several variables of reproductive success to factors population and region. We ran 50,000 replicates per model with Markov chain Monte Carlo sampler.

	Model A (region fixed)	Model B (population random)	Model C (region fixed, population random)
Fruit set	27,267.61	27,233.89	27,234.56
SO ratio	13,698.88	13,681.11	13,680.85
Seed weight	−87.61	−106.94	−102.70
Germination success	2035.95	2024.19	2023.97
Germination time (T50)	−426.38	−489.87	−489.45
Seedling emergence	3144.87	3128.42	3127.24

pollinators produced fewer fruits and had slow germination rates. In addition, the abundance of beeﬂies was positively correlated with seedling emergence (Table 3). Most pollinator variables showed significant spatial autocorrelation at different scales (see Appendix D).

### Effect of population characteristics

Population variables also varied widely across populations. Population size ranged between 120 and 2050 plants and density of *E. popovii* between 0.01 and 0.56 individuals/m<sup>2</sup> (Table 1). *E. popovii* flower density ( $3.84 \pm 3.88$  flowers/m<sup>2</sup>, range: 0.33–18.6) was much lower than that of other, co-occurring plants ( $33.24 \pm 47.44$  flowers/m<sup>2</sup>, range: 6.1–228.4) (Table 1). Annual rainfall ranged between 493 and 737 mm (Table 1).

Only the density of co-occurring flowers showed a significant correlation with reproductive output. *E. popovii* plants growing in sites with high density of co-occurring flowers produced fewer fruits (Table 3). No significant effects on fecundity were found.

As with pollinator variables, most populational variables showed significant spatial autocorrelation at different scales (see Appendix D), thus creating a complex geographic mosaic of biotic interactions and abiotic conditions.

### Discussion

Our study indicates that the reproductive output of *E. popovii* is severely limited, since less than 0.2% of the ovules produced turned into reproductive individuals. Importantly, although low seedling survival appears as the main driver of this low reproductive output. Our results also show that the reproductive stages limiting reproduction of *E. popovii* vary across the geographical range of the species.

*E. popovii* coexists with the more abundant and more widely distributed congeneric species *Erysimum mediohispanicum*. *E. mediohispanicum* blooms more profusely and produces many more ovules per individual than *E. popovii* (3535 and 839, respectively), and has a higher fruit set ( $0.82 \pm 0.03$  and  $0.67 \pm 0.14$ , respectively) (Gómez et al. 2010). The SO ratio is similar in both species ( $0.70 \pm 0.23$  in *E. mediohispanicum* and  $0.74 \pm 0.16$  in *E. popovii*) (Gómez

2005a), and seedling emergence rate is higher in *E. popovii* (0.14% seedlings per seed versus 0.09% in *E. mediohispanicum*) (Gómez 2005a). However, *E. popovii* seedling survival rate is lower than in *E. mediohispanicum* (0.06 and 0.21, respectively) (Gómez 2005a). As a result, the reproductive output of *E. popovii* (0.2% of the ovules turning into reproductive individuals) is considerably lower than that of *E. mediohispanicum* (1.1%). The low number of ovules per individual and the low seedling survival rate of *E. popovii* in comparison to *E. mediohispanicum* may contribute to explain the differences in distribution between the two species, as well as the endemic and threatened status of *E. popovii*. Ovule production and seedling survival have often been identified as drivers of reproductive outcome (Kaye 1999) and restricted distribution in endemic plants (e.g. Copete, Herranz, & Ferrandis 2008). These results are likely to apply to our short-lived species.

Many studies have demonstrated that seed weight is a good estimate of some reproductive variables, such as seed germination and seedling growth (Houssard & Escarré 1991; Navarro & Guitián 2003). However, seed weight showed no relationship with germination success, germination time or seedling emergence in *E. popovii*. Instead, we found a negative correlation between seed weight and SO ratio. This pattern suggests a trade-off between seed size and seed number, as found in many other plant species (Guo, Mazer, & Du 2010).

Germination time in *E. popovii* was similar to that of *E. mediohispanicum*, with both species yielding T50 values of about 5 days. Similar results have been reported for other *Brassicaceae* species from the Iberian Peninsula (Maselli, Pérez-García, & Aguinalgalde 1999). In annual herbaceous communities, early germinating individuals may be better competitors, as short delays in emergence may result in large differences in final biomass and fitness (Rice 1990).

We found no relationship between germination success in the laboratory and seedling emergence in the field. These results highlight the importance of assessing germination under both situations. Laboratory germination tests provide estimates of seed performance under standard conditions, but not under suboptimal, often harsh, field conditions. Some studies have shown that populations exhibiting similar levels of germination success in the laboratory, respond very differently to a wide range of suboptimal environmental

**Table 3.** Coefficient, standard error, *t* and *p* values of spatially explicit models relating several variables of reproductive success, seed quality and fecundity to pollinator and population variables, respectively.

	Fruit set			SO ratio			Germination success			Seedling emergence		
	Coeff ± SE	<i>t</i>	<i>p</i> Value	Coeff ± SE	<i>t</i>	<i>p</i> Value	Coeff ± SE	<i>t</i>	<i>p</i> Value	Coeff ± SE	<i>t</i>	<i>p</i> Value
<i>Pollinator variables</i>												
Pollinator diversity (Hurlbert's PIE)	0.017 ± 0.28	0.06	0.953	0.32 ± 0.88	0.36	0.722	−0.54 ± 1.35	−0.40	0.694	0.634 ± 0.37	1.68	0.114
Large bee (visits/h)	<0.001 ± 0.001	0.46	0.654	0.004 ± 0.006	0.62	0.545	0.005 ± 0.01	0.54	0.593	0.003 ± 0.003	1.30	0.214
Small bee (visits/h)	0.002 ± 0.003	0.55	0.588	−0.007 ± 0.01	−0.67	0.512	−0.008 ± 0.01	−0.48	0.637	−0.004 ± 0.004	−0.92	0.371
Bee fly (visits/h)	<0.001 ± 0.001	−0.81	0.429	−0.004 ± 0.004	−1.05	0.308	0.002 ± 0.005	0.41	0.685	<b>0.004 ± 0.002</b>	<b>2.83</b>	<b>0.013</b>
Low-quality pollinator (visits/h)	<b>−0.005 ± 0.002</b>	<b>−2.17</b>	<b>0.047</b>	−0.011 ± 0.01	−1.68	0.114	0.011 ± 0.01	1.08	0.297	−0.001 ± 0.003	−0.41	0.685
<i>Population variables</i>												
Reproductive plants in population	<0.001 ± <0.001	7.02	0.061	<0.001 ± <0.001	−0.79	0.44	<0.001 ± <0.001	0.908	0.379	<0.001 ± <0.001	−0.40	0.693
Population density (ind./m <sup>2</sup> )	−0.11 ± 0.083	−1.37	0.193	0.27 ± 0.26	1.05	0.308	0.084 ± 0.58	0.14	0.888	−0.053 ± 0.17	−0.29	0.769
Flowers in population	<0.001 ± <0.001	−1.48	0.16	<0.001 ± <0.001	−1.35	0.196	<0.001 ± <0.001	−0.93	0.368	<0.001 ± <0.001	0.08	0.938
Co-occurring flowers (flowers/m <sup>2</sup> )	<b>&lt;0.001 ± &lt;0.001</b>	<b>−4.61</b>	<b>&lt;0.001</b>	<0.001 ± <0.001	−0.06	0.948	<0.001 ± <0.001	−0.06	0.951	<0.001 ± <0.001	−1.63	0.124
Mean annual rainfall (l/m <sup>2</sup> )	<0.001 ± <0.001	0.98	0.343	<0.001 ± <0.001	0.215	0.833	<0.001 ± <0.001	0.61	0.552	<0.001 ± <0.001	−1.37	0.192
<i>Seedling survival</i>												
<i>Seed weight</i>												
<i>Germination time (T-50)</i>												
<i>Fecundity</i>												
	Coeff ± SE	<i>t</i>	<i>p</i> Value	Coeff ± SE	<i>t</i>	<i>p</i> Value	Coeff ± SE	<i>t</i>	<i>p</i> Value	Coeff ± SE	<i>t</i>	<i>p</i> Value
<i>Pollinator variables</i>												
Pollinator diversity (Hurlbert's PIE)	−0.166 ± 0.25	−0.66	0.522	−1 ± 0.96	−1.04	0.316	−1.15 ± 9.19	−0.12	0.902	9.162 ± 14.42	0.63	0.538
Large bee (visits/h)	0.001 ± 0.002	0.71	0.49	−0.003 ± 0.006	−0.42	0.677	0.039 ± 0.63	0.19	0.548	0.077 ± 0.097	0.79	0.446
Small bee (visits/h)	<0.001 ± 0.003	−0.09	0.926	−0.001 ± 0.011	−0.08	0.931	−0.117 ± 0.11	−1.07	0.306	−0.095 ± −0.17	−0.56	0.589
Bee fly (visits/h)	−0.001 ± <0.001	−1.12	0.287	−0.002 ± 0.004	−0.52	0.608	0.034 ± 0.04	0.84	0.418	−0.002 ± 0.057	−0.04	0.967
Low-quality pollinator (visits/h)	<0.001 ± 0.003	0.03	0.973	0.006 ± 0.007	0.84	0.414	<b>0.166 ± 0.07</b>	<b>2.38</b>	<b>0.034</b>	0.105 ± 0.195	0.33	0.745
<i>Population variables</i>												
Reproductive plants in population	<0.001 ± <0.001	−0.22	0.832	<0.001 ± <0.001	0.93	0.36	0.001 ± 0.002	0.64	0.528	−0.002 ± 0.004	−0.54	0.599
Population density (ind./m <sup>2</sup> )	−0.08 ± 0.113	−0.71	0.495	−0.038 ± 0.37	−0.10	0.92	2.7 ± 3.61	0.74	0.467	−5.862 ± 5.34	−1.09	0.296
Flowers in population	<0.001 ± <0.001	−0.13	0.895	<0.001 ± <0.001	−0.66	0.519	<0.001 ± <0.001	−0.83	0.416	<0.001 ± <0.001	0.23	0.823
Co-occurring flowers (flowers/m <sup>2</sup> )	<0.001 ± 0.002	0.43	0.678	<0.001 ± <0.001	0.32	0.751	0.015 ± 0.01	1.73	0.106	0.028 ± 0.082	0.34	0.737
Mean annual rainfall (l/m <sup>2</sup> )	<0.001 ± <0.001	0.20	0.844	<0.001 ± <0.001	1.87	0.082	<0.001 ± <0.001	1.44	0.169	<0.001 ± <0.001	−0.02	0.987

Significant models are marked with bold.



conditions (Summerfield 1972; Baskin & Baskin 2001), this is especially important in narrow endemic species which are adapted to restricted specific conditions (Pérez-García et al. 2008).

Seven of our populations showed very low (<0.01) seedling survival, which may constitute a regeneration bottleneck. These populations were not particularly small, but could suffer drastic declines over the next generations if low levels of seedling survival persist. If so, these populations could experience pollen limitation as a consequence of pollinators depositing heterospecific pollen and/or inbreeding depression as a consequence of pollinators depositing self-pollen or pollen from closely related individuals (Herrera 1987; Pflugshaupt, Kollmann, Fischer, & Roy 2002). The large differences among populations in recruitment, together with the absence of a dormant stage and the short life-cycle suggest a metapopulation structure for *E. popovii*. Given the small population sizes and the long distances between populations, together with the low dispersal ability found in other *Erysimum* species (Gómez 2007), local extinctions may become increasingly frequent and recolonization events increasingly rare.

We found no difference in reproductive output between regions. However, SO ratio, seed germination, seedling emergence and seedling survival showed spatial autocorrelation at different distances. This geographic pattern of variation in reproductive output suggests that variables affecting reproduction success are structured at different scales. The spatial variation between populations could be mediated by rainfall which usually presents high spatial correlation and germination responses have been commonly linked to particular characteristics of the precipitation regime, especially seasonal and annual variation (Venable 2007). Higher rates of seed germination would be expected to occur in regions with higher precipitation (Venable 2007). Rainfall has been shown to affect seedling emergence and survival in other Mediterranean plants (Rebollo et al. 2001). Nevertheless, we found no correlation between rainfall and reproductive output. A possible explanation for this discrepancy is that we used mean annual, instead of seasonal rainfall, which may be a stronger predictor of reproduction success (Crone & Lesica 2006). Summer drought is one of the main causes of seedling mortality in Mediterranean plants (Peñuelas et al. 2004; Harel, Holzapfel, & Sternberg 2011). Thus, rainfall occurring in summer or shortly after seedling emergence may be a stronger determinant of *E. popovii* reproductive success than annual rainfall. Additional studies will be necessary to test this hypothesis, as seasonal precipitation data were not available for individual sites in our study.

Recruitment is related to abundance, diversity and identity of pollinators in many species (Knight et al. 2005; Anderson & Johnson 2008; Gómez et al. 2010). *E. popovii* flowers are visited by a very diverse assemblage of pollinators (166 species), including large bees, small bees, beeflies, butterflies, hoverflies, beetles, and ants (Fernández et al. 2012; Fernández & Gómez 2012). We found a positive relationship

between bee fly visitation and *E. popovii* reproductive output via seedling emergence. This result may be a consequence of the high pollinating efficiency displayed by bee flies on *Erysimum*. Compared to other flower visitors, the foraging behavior of bee flies, with frequent movements between individual plants, maximizes the deposition of allogamous pollen per visit (Gómez, Perfectti, & Jordano 2011; Fernández et al. 2012). On the other hand, visitation by low quality pollinators showed a negative relationship with fruit set and germination time. This result is remarkable, given the high diversity of pollinator species encountered, and is probably related to the negative relationship between *E. popovii* fruit set and density of co-occurring flowers. In fact, density of co-occurring flowers and visitation rates of low-quality pollinators are positively correlated ( $r=0.732$ ,  $p<0.001$ ). Pollinator visitation rates in *E. popovii* are three times lower than those reported in other *Erysimum* species (Gómez et al. 2007; Fernández et al. 2012). Taken together, this evidence suggests that, in the presence of other pollen-nectar sources, high-quality pollinators tend to disregard *E. popovii* flowers. In addition, some low quality pollinators (ants) act as nectar thieves (Gómez et al. 2007), and others (small beetles) force their way into the flowers altering the appearance of the corolla. This could negatively affect subsequent visitation rates by high quality pollinators. In fact, correlation between low quality and bee fly visitation was marginally significant.

In conclusion, *E. popovii* reproductive success varies widely among populations, and this variation seems to be mostly related to local pollinator assemblage and flower community. These differences do not show a regional structure. Rather, they generate a geographic mosaic, with some populations recruiting many new individuals every year and others showing no regeneration. This pattern suggests the occurrence of a metapopulation system as described in other species (Bonet et al. 2005). Further studies exploring the connectivity between *E. popovii* populations and regions would help to evaluate the viability of this near threatened species across its entire distribution range. Because local pollinator assemblages in Mediterranean areas are known to vary among plant populations even at small geographical scales (Gómez et al. 2007; Torné-Noguera et al. 2014) our results (a geographic mosaic of reproductive output mediated by limitations in different life cycle phases) is likely to apply to other endemic Mediterranean plants with patchy distributions.

## Acknowledgments

Modesto Berbel, Beatriz Nieto, Ángela Cano, Margarita Fernández, Ana B. Romero, Miguel Ballesteros, Eva M<sup>a</sup> Cañadas and Helena Barril, helped during various stages of the research. This study was partially funded by MICINN (FPU-2006 and CGL2012-34736), MARM (078/2007), Junta de Andalucía (P07-RNM-02869 and P11-RNM-7676), and Consolider-Ingenio (CSD2008-00040) grants.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.baae.2015.02.006>.

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