



Pollination effectiveness in a generalist plant: adding the genetic component

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Summary

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Key words: effectiveness landscape, *Erysimum mediohispanicum*, generalist pollination system, multiple paternity, pollination effectiveness. • The pollination effectiveness of a flower visitor has traditionally been measured as the product of a quantity component that depends on the frequency of interaction and a quality component that measures the per-visit effects on plant reproduction. We propose that this could be complemented with a genetic component informing about each pollinator's contribution to the genetic diversity and composition of the plant progeny.

• We measured the quantity and quality components of effectiveness of most pollinator functional groups of the generalist herb *Erysimum mediohispanicum*. We used 10 microsatellite markers to calculate the genetic component as the diversity of sires among siblings and included it into the calculation of the pollination effectiveness.

• Functional groups varied in the quantity and quality components, which were shown to be decoupled. Functional groups also differed in the genetic component. This component changed the estimates of pollination effectiveness, increasing the differences between some functional groups and modifying the pollination effectiveness landscape.

• We demonstrate that including the genetic component in the calculation of the pollination effectiveness may allow a more complete quantification of the contribution of each pollinator to the reproductive success of a plant, providing information on its mating patterns and long-term fitness.

Introduction

The pollination effectiveness of a flower visitor estimates its contribution the plant's reproductive success (Ne'eman et al., 2010; Schupp et al., 2017). An accurate assessment of its magnitude is crucial to understand the ecology and evolution of plant-pollinator interactions (Wilcock & Neiland, 2002). Determining the variation in pollination effectiveness may help to identify which flower visitors act as true pollinators against those providing very low benefit to their host plants or even being involved in floral larceny (Inouye, 1980; Herrera, 1987; Maloof & Inouye, 2000; Rodríguez-Rodríguez et al., 2013; Armbruster, 2014), to ascertain which pollinators contribute most to seed yield in crops (Rader et al., 2012), or to improve the reliability and predictive power of network analyses in plant-pollinator interaction studies (Ballantyne et al., 2015, 2017; Tur et al., 2016; Willcox et al., 2017). From an evolutionary point of view, it may provide important information on the strength of the selective pressures exerted by each flower visitor (Gómez & Zamora, 1999; Van Der Niet et al., 2014; Ollerton et al., 2015).

Pollination effectiveness is composed of two components: a quantity component QNC and a quality component QLC (Herrera, 1987, 1989; Schupp *et al.*, 2017). The QNC, measures the number of pollen grains deposited on a stigma by the population

of a given pollinator. This component depends on the frequency of interaction (Fig. 1) which correlates with the importance of pollinators in some plant communities (Ballantyne et al., 2017), and has been considered a good predictor of the total effect that pollinators have on plant reproduction (Vázquez et al., 2005, 2015). However, this relationship is not universal because not always the most abundant pollinators are the most effective (Perfectti et al., 2009; King et al., 2013), implying that information on the per-visit quality of the interaction is needed to get an accurate evaluation of the role played by each pollinator species on plant reproduction. The QLC is the probability of an ovule producing a viable seed after a single visit to a flower (Fig. 1; Schupp et al., 2017). This component has been determined using different surrogates corresponding to the diverse phases of pollination after pollen deposition such as the proportion of pollen grains developing pollen tubes (Alonso et al., 2011). However, more complete estimates of QLC are those considering postpollination stages of the interaction, such as the proportion of fruit set (Brittain et al., 2013) and, most importantly, the production of seeds or even seedlings after a single visit (Mayfield et al., 2001; Rodríguez-Rodríguez et al., 2013; Bruckman & Campbell, 2014; Wang et al., 2017).

Although these estimates provide useful information about the effects of pollinators on the reproduction of plants, they

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Fig. 1 Processes involved in the pollination effectiveness. At the top, grey boxes highlight each component of pollination effectiveness as quantified in this work. Quantity component: A, number of insect visits to a plant; B, per-plant number of contacted flowers. Quality component: C, fruiting probability; D, proportion of ovules setting seeds; E, germination success. Genetic component: F, per-fruit diversity of sires. Coloured symbols indicate the identity of each pollen donor. Failure in ovule fertilization and seed germination is denoted with the ' \times ' symbol.

overlook some other subtle processes that may influence the evolution of plant populations. Specifically, several features of pollinators such as the plant visitation pattern, carryover capacity or their ability to promote self-pollination may result in the transference of mixed pollen loads differing in genetic composition (Bernasconi, 2003; Pannell & Labouche, 2013; Barrett & Harder, 2017; Rhodes et al., 2017). Genetically diverse pollen loads provide the appropriate environments for pollen competition (Marshall & Folsom, 1991; Marshall et al., 2007), influencing the quality component of effectiveness in multiple ways. For example, it can increase the number of fertilized ovules by enhancing the germination of compatible pollen (Montalvo, 1992) and the production of more vigorous offspring (Gómez, 2000; Herrera, 2000; Rostás et al., 2018). Moreover, it also can result in multiple paternity within the same fruit (Ellstrand, 1984). Multiple paternity can mitigate the effects of pollen limitation when seed set is limited by the quality rather than the quantity of pollen (Aizen & Harder, 2007). It also allows female plants to choose among diverse male gametophytes through post-zygotic mechanisms (Pannell & Labouche, 2013) and to allocate more resources to multiply sired fruits, resulting in more and larger seeds with better competitive abilities and germination probabilities (Marshall & Ellstrand, 1986). Multiple paternity reduces biparental inbreeding among nearby halfsiblings in those plants with limited seed dispersal (Griffin & Eckert, 2003). Finally, multiple paternity increases the likelihood of favourable combinations of genotypes within the progeny (Paschke et al., 2002), offering the opportunity to hedge bets by spreading the risk of failure mating (Cohen, 1966; Simons, 2011; Garcia-Gonzalez et al., 2014; Yasui & Garcia-Gonzalez, 2016). Despite the many benefits for plant reproductive success, the ability of pollinators to cause withinfruit multiple paternities traditionally has been overlooked when estimating pollination effectiveness (Rhodes et al., 2017).

We propose that the pollinator-mediated genetic composition of a plant's progeny can be considered a new component for the calculation of pollination effectiveness. This genetic component (GNC) gathers information about relevant patterns of pollen flow for plant reproduction such as the distance of pollination (Price & Waser, 1979; Castilla et al., 2017), immigrant pollen flow (Dudash, 1990) or patterns of assortative mating (Kennedy et al., 2006). But perhaps the simplest and less controversial information derived from this component is the diversity of sires (Rhodes et al., 2017). When the GNC is calculated as the diversity of sires, the genetically oriented effectiveness can be interpreted as the diversity of plant genotypes produced by the population of a given pollinator. In this way, the genetic component gives a more comprehensive estimate of effectiveness that takes into account the evolutionary potential of the interaction.

In the present study we explore the consequences of considering the genetic component on the final assessment of pollination effectiveness. We use as model system Erysimum mediohispanicum (Brassicaceae), a Mediterranean herb displaying a generalist pollination system (Gómez et al., 2009b, 2014). In generalist systems, pollinators differ not only in their abundance at flowers but also in important functional traits that may affect their pollination effectiveness, such as foraging behaviour, floral fidelity or the morphological fit to the reproductive structures of flowers (Brosi & Briggs, 2013). We performed a comprehensive estimation of the QNC and QLC of most insect species visiting the flowers of *E. mediohispanicum*. Using paternity analyses we characterized the GNC as the within-fruit diversity of sires produced after a single pollination event. We hypothesize that including the GNC into the standard effectiveness framework will provide information on the role of each pollinator to the mating portfolio of plants (Barrett & Harder, 2017) and will give a more complete estimate of its pollination effectiveness. We argue that future studies on pollination effectiveness should consider genetic aspects of the progeny to inform not only about the early demographic consequences of the interaction but also to include other aspects as the contribution of each pollinator to the mating patterns and the long-term fitness of the plant.

Materials and Methods

Study system and area of study

Erysimum mediohispanicum Polatschek (Brassicaceae) is a biennial plant endemic to montane areas of the Iberian Peninsula. Individuals grow vegetatively for 2-3 yr and flower from May to July, developing one to eight flowering stalks. Flowers develop sequentially, from bottom to top along the stalks. These are hermaphrodite and partially self-compatible (Abdelaziz et al., 2014). Nevertheless, this species requires the assistance of pollinators to produce full seed set (Gómez, 2005). Its pollination system is highly generalist, with several hundred insect species visiting their flowers (Gómez et al., 2009a,b) which can be clustered into 23 functional groups (see Supporting Information Table S1; Valverde et al., 2016b). Following Fenster et al. (2004), we considered a functional group as a group of pollinators exerting similar selective pressures on flower traits. Functional groups are internally coherent based on pollinator similarities in flower handling, size or morphological fit with the flower (see example in Table S2).

This study was conducted between 2010 and 2013 at the Sierra Nevada Protected Area (south-east Spain), in a mediumhigh mountain Mediterranean-type ecosystem. The study was performed in two localities within the area of distribution of *E. mediohispanicum*: a natural population situated in an open woodland ($37^{\circ}8'07''$ N, $3^{\circ}21'71''$ W, 1723 m above sea level (asl), 2010 and 2011; see Valverde *et al.*, 2016a for more details), and at the Hoya de Pedraza Botanical Garden ($37^{\circ}6'41''$ N, $3^{\circ}26'13''$ W, 1950 m asl, 2011–2013).

Quantity component

We assessed the QNC of each functional group as its per-flower visitation rate. For this, we considered two multiplicative subcomponents: plant visitation rate (number of insects visiting a plant during 5 min) and the per-plant number of contacted flowers (Fig. 1). The QNC was quantified in 2010 and 2011 at the natural population. The first subcomponent was obtained by surveys of 5 min on 200 focal plants (100 per year in 2010 and 2011). We performed these surveys daily along the whole flowering season and from one to four times per day within the time frame 11:00 h and 18:00 h (Valverde et al., 2014, 2016b). The second subcomponent was recorded on randomly selected insects foraging on the flowers of E. mediohispanicum. Some insects stayed at a plant for > 5 min. In those cases, we used the average flower handling time of the corresponding functional group to estimate the per-plant number of contacted flowers in 5 min. Handling times were measured on some foraging bouts using a digital stopwatch.

Quality component

We quantified the QLC as the probability of an ovule of producing a viable seed after a single visit to a flower. For this we considered three multiplicative subcomponents: fruit set (fruiting probability), seed set (proportion of ovules setting seeds) and germination success (Fig. 1). The QLC was quantified from 2011 to 2013 at the Hoya de Pedraza Botanical Garden through single-visit experiments. We used 50 flowering plants from random seeds collected from the population in 2010 and grown in nurseries containing the same soil and water supply. These plants were excluded from pollinators using fine mesh tents (*c.* 160-µm mesh aperture; BugDorm-2120; MegaView Co. Ltd, Taichung, Taiwan). Each time a plant was exposed to flower visitors, each visitor was allowed to contact up to three virgin flowers. After a visit, the contacted flowers were marked with coloured cotton threads. We photographed each visitation event to identify the insect species.

We recorded the proportion of those flowers setting fruit. Afterwards, we recorded the proportion of ovules setting seeds (seed set) and the proportion of fertilized ovules aborted (abortion rate) of each of the produced fruits. Under a stereo microscope, fertilized ovules are distinguished from unfertilized ovules for being bigger and darker. Viable seeds have a uniform shining brown colour, and are healthy in appearance, whereas aborted seeds are wrinkled and of smaller size. Finally, we assessed the germinability of those seeds produced by the four most effective functional groups (see Results) using all seeds from the fruits yielding more than four seeds (long-tongued beeflies = 18 fruits; long-tongued large bees = 50; short-tongued large bees = 13; short-tongued medium bees = 8). Seeds were sown in seedling trays in a glasshouse under homogeneous light and water supply, and their germination success recorded during the following two months.

In addition, we performed three pollination treatments in each of the experimental plants: an outcrossing treatment, in which pollen from three individuals was added to a virgin stigma; a selfing treatment, in which pollen from the same flower was added to a stigma; and a procedural control, in which no pollen was added. In all treatments petals were cut off to prevent further visits. All treatments were performed at least twice along the flowering stalk to account for the possible effects of flower position caused by resource allocation (Wesselingh, 2006; Collin *et al.*, 2009).

Genetic component

For long-tongued beeflies and short- and long-tongued large bees (the three functional groups showing the highest QNC \times QLC values), we identified the sire of each seed produced after a single visit to a flower. This allowed quantification of three genetic parameters: the per-fruit diversity of sires, the pollination distance and the per-fruit proportion of seeds sired by immigrant pollen.

During 2012, we set up two plots of plants at the Hoya de Pedraza Botanical Garden that served as pollen donors. Each plot consisted of 49 plants in a regular 7×7 grid with 0.5-m spacing between plants (Fig. S1). Plots were 10.3 m away from each other and > 50 m from the nearest *E. mediohispanicum* individuals growing naturally. To ensure homogeneous light conditions and avoid

interference with other plant species, the plots were placed in an open field on a bare soil. Pollen donors originated from randomly chosen seeds from the natural population in 2010. As mother plants we used 20 plants used previously to calculate the QLC. Similar to the QLC experiment, these were kept isolated from pollinators. Each time they were exposed, they were randomly placed at specified spots located 0.5 m from the border of any plot (Fig. S1). The fruits produced after single visits to virgin flowers were collected and their seeds planted as explained above. Maternal plants, pollen donors and at least four seeds (seedlings) per fruit from a set of randomly chosen fruits were genotyped using 10 unlinked nuclear microsatellites loci (Muñoz-Pajares *et al.*, 2011).

We performed paternity analyses using the exclusion method in CERVUS v.3.0 (Marshall *et al.*, 1998). After 50 000 simulations we calculated strict and relaxed thresholds (95% and 80%, respectively) for the likelihood-odds ratio scores (LOD) and for the LOD difference between the two most likely pollen donors (Δ). We assumed an error rate of 0.20 based on the observed maternal–offspring genotype mismatches (Table S3). We set the proportion of genotyped males to 0.88 as we successfully genotyped 86 of the 98 pollen donors. Finally, based on our data, the proportion of typed loci was set to 0.93. Seeds with fewer than five loci genotyped were not considered.

In this way, a pollen donor was correctly assigned as a sire if LOD and Δ were higher than their respective strict thresholds, and unassigned if LOD was lower than the relaxed threshold. Different LOD and Δ scores than the latter may indicate allelic mismatches between the seed and the potential pollen donor. In such cases we checked if such mismatches were due to null alleles, otherwise such a pollen donor was declined as the sire. Because we could not successfully genotype all pollen donors, unassigned seeds may have been sired by either nongenotyped individuals from the plots or by immigrant pollen. To distinguish between these two possibilities, we used the GENECLASS2 software (Piry et al., 2004) to calculate the likelihood of the paternal fraction of each seed genotype belonging to the genetic pool of the plots. For this calculation, we simulated gamete genotypes from the plots using the allelic frequencies from the genotyped pollen donors (Paetkau et al., 1995, 2004).

We estimated the per-fruit diversity of sires using the Hurlbert's Probability of Interspecific Encounter (PIE; Hurlbert, 1971). Here, this index measures the probability that two randomly sampled seeds from a fruit have two different sires. It ranges from 0, when progeny is formed by full siblings (all share the same sire) to 1, when progeny is formed by half-siblings (each seed has a different sire). For those seeds sired by nongenotyped pollen donors, we considered a sole dummy sire and for those sired by immigrant pollen a different dummy sire. Because this may underestimate the contribution of nongenotyped sires, we omitted those fruits producing more than two seeds from dummy sires. We cross-validated this measure with the correlation of paternity ($F_{\rm S}$; Hardy *et al.*, 2004) –the probability of paternal identity among siblings– using KINDIST (Robledo-Arnuncio *et al.*, 2007).

In order to calculate the pollination distance we used the spatial location of sires and mother plants at the moment of the pollination event. Finally, the per-fruit proportion of immigrant pollen was obtained directly from the estimated number of seeds assigned to dummy sires outside any of the plots (> 50 m). Although these two aspects are part of the genetic component, we considered only the diversity of sires in its calculation.

Pollination effectiveness

We first calculated the pollination effectiveness for each pollinator functional group by multiplying the quantity and quality components (QNC \times QLC). To estimate the mean and the 95% confidence interval of this product we used the bootstrap-based method of Reynolds & Fenster (2008) by means of 100 simulations, each consisting in 5000 bootstrap resamples of the original data. We plotted the effectiveness values in a pollination effectiveness landscape, a 2D plot that connect by means of isoclines all combinations of QNC and QLC yielding the same effectiveness values (Schupp *et al.*, 2017). Because of the high consistency among simulations (see Results), we randomly chose one to be represented in the effectiveness landscape.

Afterwards, we recalculated the pollination effectiveness of long-tongued beeflies and short- and long-tongued large bees by incorporating GNC as an additional multiplicative component (QNC \times QLC \times GNC). These results also were represented in a pollination landscape plotting QNC \times QLC against GNC.

Statistical analyses

We compared QNC and QLC among functional groups using generalized mixed models and the GLMMADMB (Fournier *et al.*, 2012) and LMER (Bates *et al.*, 2015) packages in R. For the plant visitation rate, we used the data of those plants with more than five visits and used a zero-inflated Poisson distribution, whereas for the per-plant number of contacted flowers we used a standard Poisson distribution. In both cases we clumped data from the years 2010 and 2011 because of the low interannual variation in pollinator assemblages (Valverde *et al.*, 2016b) and corrected by the number of open flowers by setting its natural logarithm as an offset variable in the statistical models. The number of open flowers per day was assessed by periodically recording the number of open flowers and using local polynomial functions to estimate each plant's flowering curve (Valverde *et al.*, 2016b).

Fruit set was analysed using a binomial distribution, whereas the seed set and the abortion rate were analysed using a quasibinomial and logit link function. Because germination success was 100% in all treatments, we did not analyse this subcomponent. In all cases, we used plant identity as random variable and *post hoc* multiple comparisons using Tukey's range test and Bonferroni correction (R/MULTCOMP package; Hothorn *et al.*, 2008).

The per-fruit diversity of sires, the correlation of paternity and the per-fruit proportion of seeds sired by immigrant pollen were compared between functional groups using the two-sample permutational Wilcoxon test (R/PERM package; Fay & Shaw, 2010). We used this test due to the small sample size after clumping genotypes by fruit. Within-plot pollination distances (0–4.5 m) were compared using the Kolmogorov–Smirnov *U*-test.

Data accessibility

Raw data are available at 10.6084/m9.figshare.7692350.

Results

Quantity component

We recorded 2022 plant visits (889 visits, 21 735 min of survey in 2010; 1133 visits, 22 240 min in 2011). Of these visits, 27% were performed by long-tongued beeflies, which were significantly the most common visitors (|z| = 2.65-9.63, P < 0.005; Fig. 2a; Tables S4, S5). The next most common visitors were small beetles, at 23.6% of the visits, and short-tongued large bees, at 15.9%. By contrast, the per-plant number of contacted flowers was more evenly distributed among functional groups, being highest for long-tongued beeflies (Fig. 2b; Table S4) but not significantly different from long-tongued large bees and short-tongued large bees (|z| = 0.42-2.04, P=1; Table S6). The rest of the functional groups visited on average fewer than three flowers per plant.

Quality component

We obtained data from 947 flowers (Table S4). The outcrossing treatment performed better for all subcomponents, being significantly higher than all functional groups for the seed set (|z| = 4.43-20.22, P < 0.05; Fig. 2c; Tables S4, S7). However,



Fig. 2 Quantity and quality subcomponents of pollination effectiveness of the pollinator assemblage of *Erysimum mediohispanicum*. Quantity subcomponents: (a) plant visitation rate; (b) per-plant number of contacted flowers. Quality subcomponents: (c) fruit set; (d) seed set. Mean values after 5000 bootstrap resamples are shown for pollination treatments (grey circles), the four most effective pollinator functional groups (white circles) and the rest of the pollinator functional groups (black circles). Error bars represent the 95% confidence interval of the mean. Pollinator functional groups are ordered following their effectiveness calculated as the product of the quantity and the quality components. Letters denote significant differences between groups after *post hoc* multiple comparisons. Silhouettes correspond to the four most effective pollinator functional groups.

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the selfing treatment and the procedural control showed the lowest values in all the subcomponents (fruit set < 0.29, seed set < 0.07). Long-tongued large bees, short-tongued large and medium-sized bees and long-tongued beeflies stand out in quality (Fig. 2c,d). Fruit set was higher in short- and longtongued large bees and in large hoverflies (Fig. 2c; Tables S4, S8), with values similar to the outcrossing treatment (|z| = 1.35 - 2.47, P = 1). Seed set was significantly higher in flowers pollinated by short- and long-tongued large bees than the rest of functional groups (|z| = 3.56-9.48, P < 0.03; Fig. 2d; Tables S4, S7). Abortion rate was low and similar to the outcrossing treatment in all functional groups except in large hoverflies (Fig. S2).

Genetic component

A total of 180 seeds (36 fruits), 20 mother plants and 86 potential pollen donors were successfully genotyped for at least five loci. Paternity analyses assigned a sire to 121 (62%) seeds (LOD > 4.48; Δ > 1.48; Table S9) from which five presented allelic mismatches with the alleged sire. This led to 64 unassigned seeds from which 32 were assigned to a parent after checking for allelic mismatches, and 32 were kept unassigned. From the latter, 21 were inferred to have been sired by immigrant pollen. All this resulted in 82% of the seeds assigned to a sire, 6% being sired by a nongenotyped pollen donor from the plot, and 12% being sired

by immigrant pollen. We did not find any evidence of selfed seeds.

The diversity of sires was significantly higher in those fruits produced after a visit of long-tongued beeflies than after a visit of long-tongued large bees (|z| = 2.21, P < 0.03; Fig. 3a; Tables S4, S10). This pattern was validated by the correlation of paternity, which was significantly higher in long-tongued large bees $(F_{\rm S} = 0.56 \pm 0.33)$ than in long-tongued beeflies $(F_{\rm S} = 0.20 \pm$ 0.24; |z| = 2.68, P = 0.007). Most pollinations occurred within plot (80-82.5%, Table S11), followed by immigrant pollen (10.5-12.4%) and between plots (5.2-8.9%). The within-plot pollination distances of the three functional groups differed from the potential pollination distances (D=0.47-0.55, P<0.001). Long-tongued large bees moved pollen the shortest distances (mean = 1.15 m; Fig. 3c; Table S11), which differed significantly from the pollen-moving distances of short-tongued large bees (mean = 1.92 m; D = 0.29, P = 0.049). Finally, the per-fruit proportion of seeds sired by immigrant pollen did not differ significantly among any pair of functional groups (|z| = 0.11-0.41; *P*>0.683; Table S10).

Pollination effectiveness

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Our estimates of the pollination effectiveness were highly consistent among simulations, both with and without the genetic component (CV < 4.56%; Table S12). The product of QNC and

Fig. 3 Genetic subcomponents of the three most effective pollinator functional groups of Erysimum mediohispanicum. (a) Per-fruit diversity of sires, calculated as the Probability of Interspecific Encounter (PIE). Letters denote significant differences between groups after post hoc multiple comparisons. (b) Per-fruit proportion of seeds sired by immigrant pollen. Circles in (a) and (b) represent observed values. (c) Pollination distances, relative frequencies of pollen dispersal calculated as the distance between the mother and sire plants of each genotyped seed. We differentiated pollen coming from the same plot (0.5–4.5 m), from the other plot (11-18 m), and immigrant pollen (>50 m). Silhouettes refer to long-tongued beeflies, short-tongued large bees and longtongued large bees (as depicted in Fig. 2).



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© 2019 The Authors New Phytologist © 2019 New Phytologist Trust QLC showed that long-tongued beeflies were the most effective pollinators of *E. mediohispanicum* (Table 1; Fig. 4a), due mainly to the QNC, which was eight times greater than that of the second most effective pollinators. The next most effective pollinators were short- and long-tongued large bees, which showed similar values of pollination effectiveness (Table 1; Fig. 4a). Short-tongued medium-sized bees had a slightly lower value of effectiveness than the three previous groups of pollinators. The rest of groups showed lower values of effectiveness (<0.38), particularly butterflies, small and large hoverflies, and large flies (<0.01; Table S12; Fig. 4a).

The inclusion of GNC increased the differences between functional groups in effectiveness. Whereas effectiveness of

long-tongued beeflies was four times higher than the effectiveness of the other two functional groups when considering only QNC × QLC, the differences between long-tongued beeflies and long-tongued large bees increased to >6.5 times after including GNC into the calculation of pollination effectiveness (Table 1). Most important, although short- and longtongued large bees showed the same effectiveness without GNC, the inclusion of this component reduced the effectiveness of long-tongued large bees in relation to that of shorttongued large bees (Table 1). As a consequence, the pollination effectiveness of long-tongued large bees (0.60×10^{-2}) was lower than the 2.5th percentile of short-tongued large bees (0.69×10^{-2}).

Table 1 Pollination effectiveness of the three most effective pollinator functional groups of Erysimum mediohispancum.

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Functional group	QNC	QLC	GNC	$QNC \times QLC (10^{-2})$	$QNC \times QLC \times GNC (10^{-2})$	
Long-tongued beeflies Short-tongued large bees	$0.69 \pm 0.03 \\ 0.08 \pm 0.01$	0.08 ± 0.01 0.17 ± 0.02	0.76 ± 0.23 0.72 ± 0.21	5.16 (3.91–6.57) 1.37 (1.04–1.77)	3.93 (2.79–5.25) 0.99 (0.69–1.35)	
Long-tongued large bees	$\textbf{0.08} \pm \textbf{0.01}$	$\textbf{0.16} \pm \textbf{0.01}$	$\textbf{0.47} \pm \textbf{0.34}$	1.26 (0.98–1.59)	0.60 (0.38–0.85)	

Effectiveness components: QNC, quantity component; QLC, quality component; GNC, genetic component. These values correspond to the mean and standard deviation of one simulation. Pollination effectiveness values before (QNC \times QLC) and after including the genetic component (QNC \times QLC \times GNC) correspond to the mean and the 2.5 and 97.5th percentiles of the estimates obtained in 100 simulations, respectively.



Fig. 4 Pollination effectiveness landscape of the pollinator assemblage of *Erysimum mediohispanicum*. (a) Landscape facing the quantity (QNC) against the quality (QLC) components. The lower panel zooms into those functional groups presenting the lowest values of effectiveness. (b) Landscape facing the product of the quantity and the quality components (QNC \times QLC) against the genetic component (GNC). Isoclines depict same values of effectiveness. Error bars represent the mean ± 2 SD (*c.* 95% CI) of the estimated components. Functional groups marked with '*' only show the mean value.

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Discussion

The decoupling of the quantity and quality components of effectiveness

Our study reveals that the pollination effectiveness (quantity times quality components; QNC × QLC) of the four major functional groups of pollinators, that is, long-tongued beeflies, short- and long-tongued large bees, and short-tongued medium bees, accounted for the 94% of the effectiveness, indicating that most of the reproduction of *Erysimum mediohispanicum* in the natural population under study was carried out by these pollinators. This suggests that the degree of generalization of *E. mediohispanicum* strongly shrinks when considering the effectiveness of pollinators, a phenomenon previously reported in other systems (Herrera, 1989; Gómez & Zamora, 1999; Sahli & Conner, 2007; Robertson & Leavitt, 2011; Watts *et al.*, 2012).

By showing different abundance at flowers and disparate foraging and flower-handling behaviours (see Notes S1; Fig. S3, for a detailed discussion), the pollinator functional groups of E. mediohispanicum differed widely in effectiveness. Thus, longtongued beeflies was the most effective functional group due mainly to its high QNC. In fact, it has been shown that beeflies may affect not only individual plants but also the structure of the E. mediohispanicum pollination networks, increasing their connectivity and decreasing their functional specialization (Gómez et al., 2011; Valverde et al., 2016b). On the other hand, the effectiveness of the second and third most effective functional groups (short- and long-tongued large bees) was mostly due to their QLC. Long-tongued large bees handle flowers very fast and with high accuracy, and perform long flights from and to outside the population, indicating that they may be key in mediating the gene flow among populations (but see discussion in the following section). Short-tongued large bees were almost exclusively represented by Lasioglossum xanthopus. This bee has strong fidelity for the flowers of *E. mediohispanicum*, a behaviour surely favouring a high amount of conspecific pollen deposition. Nevertheless, the abundance of *E. mediohispanicum* pollinators varies strongly at different spatial (Gómez et al., 2008, 2009b, 2010, 2014) and temporal scales (Valverde et al., 2016b). Consequently, the effectiveness of those functional groups that are based mainly on their QNC, such as beeflies, will surely vary largely between both plant populations and flowering periods, demonstrating that the pollination effectiveness of E. mediohispanicum flower visitors is context-dependent.

Altogether, our results indicate that QNC and QLC are decoupled, a pattern that has been reported previously for other systems (Schemske & Horvitz, 1984; Mayfield *et al.*, 2001; Ollerton *et al.*, 2015). According to the Stebbins' most effective pollinator principle, plants should evolve traits to attract mostly those pollinators transferring pollen most effectively (Stebbins, 1970). Consequently, in specialized systems an association between the quantity and quality of most effective pollinators would be expected. Conversely, as shown in Fig. 4(a), such an association is missing in our study system. The decoupling of these two

components could be a major feature of most generalist plant species (Mayfield *et al.*, 2001; Perfectti *et al.*, 2009).

The genetic component of effectiveness

We have characterized some genetic aspects of the progeny produced after a single visit by means of a paternity-based method. Although there exist alternative approaches offering the same or similar information (e.g. correlation of paternity; Ma et al., 2018), paternity-based methods are not sensitive to small sample sizes (Burczyk & Chybicki, 2004) and directly reflect the effective pollen flow (Wang, 2014; Castilla et al., 2017; Rhodes et al., 2017). This information allows characterization of the plant's mating system and the contribution of each pollinator to it (Barrett & Harder, 2017). For example, the absence of selfed seeds and the low seed set in the selfing treatment suggest the existence of mechanisms favouring allogamy against autogamy in E. mediohispanicum, from what we can conclude that most seed abortions observed in some functional groups, such as small hoverflies, could be due to the deposition of pollen of low quality (i.e. self pollen).

Effective pollen flow in natural populations is influenced by the spatial genetic structure of pollen donors (Griffin & Eckert, 2003), at-site environmental conditions (DiLeo et al., 2014) and the foraging behaviour of pollinators. However, our experimental plots lacked any genetic structure and had homogeneous environmental conditions, meaning that the measured diversity of sires truly reflects disparities in the intrinsic pollen carryover abilities among pollinators. Long-tongued beeflies produced the most genetically diverse progeny, suggesting high rates of carryover. On the contrary, the less genetically diverse progeny produced by long-tongued large bees may actually reflect their lower carryover ability. This functional group also showed the most truncated pollen dispersal over short distances, a pattern that contrasts with the occasional long flights performed by these insects in wild populations. Such disassociation is probably due to both most flights being carried out between nearby plants and most transferred pollen being collected in the previously visited plant. In this sense, our study suggests that insects performing longer flights are not necessarily the most effective at moving pollen long distances. However, because plants in our experimental plots had a regular spatial distribution and pollinators respond differently to the spatial distribution of resources (Ohashi & Thomson, 2009; Lander et al., 2013), these results must be interpreted with caution.

Deciding which genetic aspect to use as a new genetic component of the pollination effectiveness is not trivial because their benefits for plant fitness will depend on the system (Price & Waser, 1979; Dudash, 1990; Becker *et al.*, 2006). In pollination generalist plants, like the one studied here, the diversity of sires may be beneficial mostly when environmental conditions vary spatiotemporally. In such cases, genetically diverse progenies may work as a bet-hedging strategy (Cohen, 1966; Simons, 2011; Yasui & Garcia-Gonzalez, 2016), offering to the mother plant the opportunity of producing favourable combinations of genotypes facing environmental changes (Karron & Marshall, 1993;

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Yasui & Garcia-Gonzalez, 2016). In addition, in plants with limited seed dispersal, such as *E. mediohispanicum* (Gómez, 2007; Valverde *et al.*, 2016a), genetically diverse progeny can reduce the risk of biparental inbreeding among nearby dispersed siblings (Griffin & Eckert, 2003).

The inclusion of a genetic component (GNC), calculated as the per-fruit diversity of sires, entailed substantial changes in the among-functional groups variation in effectiveness. Specifically, the difference between long-tongued beeflies and the other functional groups increased, whereas the difference in effectiveness between short-tongued and long-tongued large bees became significant. This suggests that the relative importance of pollinators for plant fitness may switch when taking into account their contribution to the genetic diversity of the progenies. The consequences of considering GNC depend, however, on the QNC × QLC baseline. Because QNCs tend to vary in space and time in our study system (Gómez et al., 2009b; Valverde et al., 2016b), we expect that the relative importance of GNCs will be context-dependent. Moreover, because the genetic diversity of pollen loads may vary geographically (Robledo-Arnuncio et al., 2014) GNC also may be itself context-dependent.

Conclusions

Our study unveils interesting properties of the generalist pollination systems. In these types of systems, the variability in the QNC and QLC may entail a reduction in the number of pollinators contributing significantly to plant reproduction, drawing attention to the importance of properly defining the level of generalization. Moreover, the absence of association between those two components reveals a deviation from the expectations of the Stebbin's most effective pollinator principle, a pattern likely characterizing most generalist pollination systems. On top of this, we have demonstrated that pollinators may differ not only in the number of viable seeds produced after a single visit, but also in their contribution to the genetic composition of the progeny. The consideration of genetic aspects of the progeny produced by a given pollinator adds information about its contribution to plant mating patterns and gives a more comprehensive insight of its effectiveness. We encourage future studies to dig deeper into the nature of this GNC in order to better understand plant-pollinator interactions.

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Author contributions

JV, FP and JMG conceived the idea; JV designed, collected and analysed data, and led the writing; and all coauthors contributed to further revisions.

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References

- Abdelaziz M, Muñoz-Pajares AJ, Berbel M, Perfectti F, Gómez JM. 2014. Association between inbreeding depression and floral traits in a generalistpollinated plant. *Journal of Evolutionary Biology* 27: 2495–2506.
- Aizen MA, Harder LD. 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology* 88: 271–281.
- Alonso C, Herrera CM, Ashman T. 2011. A piece of the puzzle: a method for comparing pollination quality and quantity across multiple species and reproductive events. *New Phytologist* 193: 532–542.
- Armbruster WS. 2014. Floral specialization and angiosperm diversity: phenotypic divergence, fitness trade-offs and realized pollination accuracy. *AoB Plants* 6: plu003.
- Ballantyne G, Baldock KCR, Rendell L, Willmer PG. 2017. Pollinator importance networks illustrate the crucial value of bees in a highly speciose plant community. *Scientific Reports* 7: 8389.
- Ballantyne G, Baldock KCR, Willmer PG. 2015. Constructing more informative plant–pollinator networks: visitation and pollen deposition networks in a heathland plant community. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 282: 20151130.
- Barrett SCH, Harder LD. 2017. The ecology of mating and its evolutionary consequences in seed plants. Annual Review of Ecology, Evolution, and Systematics 48: 135–157.
- Bates D, Mächler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48.
- Becker U, Reinhold T, Matthies D. 2006. Effects of pollination distance on reproduction and offspring performance in *Hypochoeris radicata*: experiments with plants from three European regions. *Biological Conservation* 132: 109–118.
- Bernasconi G. 2003. Seed paternity in flowering plants: an evolutionary perspective. *Perspectives in Plant Ecology, Evolution and Systematics* 6: 149–158.
- Brittain C, Williams NM, Kremen C, Klein A-M. 2013. Synergistic effects of non-Apis bees and honey bees for pollination services. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 280: 20122767.
- Brosi BJ, Briggs HM. 2013. Single pollinator species losses reduce floral fidelity and plant reproductive function. *Proceedings of the National Academy of Sciences*, USA 110: 13044–13048.
- Bruckman D, Campbell DR. 2014. Floral neighborhood influences pollinator assemblages and effective pollination in a native plant. *Oecologia* 176: 465–476.
 Burczyk J, Chybicki IJ. 2004. Cautions on direct gene flow estimation in plant
- populations. *Evolution* 58: 956–963.
- Castilla AR, Pope NS, O'Connell M, Rodriguez MF, Treviño L, Santos A, Jha S. 2017. Adding landscape genetics and individual traits to the ecosystem function paradigm reveals the importance of species functional breadth. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 114: 12761–12766.

Cohen D. 1966. Optimizing reproduction in a randomly varying environment. *Journal of Theoretical Biology* 12: 119–129.

- Collin CL, Penet L, Shykoff JA. 2009. Early inbreeding depression in the sexually polymorphic plant *Dianthus sylvestris* (Caryophyllaceae): effects of selfing and biparental inbreeding among sex morphs. *American Journal of Botany* **96**: 2279–2287.
- DiLeo MF, Siu JC, Rhodes MK, López-Villalobos A, Redwine A, Ksiazek K, Dyer R. 2014. The gravity of pollination: integrating at-site features into spatial analysis of contemporary pollen movement. *Molecular Ecology* 23: 3973–3982.
- **Dudash MR. 1990.** Relative fitness of selfed and outcrossed progeny in a selfcompatible, protandrous species, *Sabatia angularis* L. (Genatianaceae): a comparison in three environments. *Evolution* 44: 1129–1139.
- Ellstrand NC. 1984. Multiple paternity within the fruits of the wild radish, *Raphanus sativus. The American Naturalist* 123: 819–828.
- Fay MP, Shaw PA. 2010. Exact and asymptotic weighted logrank tests for interval censored data: the interval R package. *Journal of Statistical Software* 36: 1–34.
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35: 375–403.
- Fournier DA, Skaug HJ, Ancheta J, Ianelli J, Magnusson A, Maunder MN, Nielsen A, Sibert J. 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optimization Methods and Software* 27: 233–249.
- Garcia-Gonzalez F, Yasui Y, Evans JP. 2014. Mating portfolios: bet-hedging, sexual selection and female multiple mating. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 282: 20141525.
- Gómez JM. 2000. Effectiveness of ants as pollinators of *Lobularia maritima*: effects on main sequential fitness components of the host plant. *Oecologia* 122: 90–97.
- Gómez JM. 2005. Non-additive effects of herbivores and pollinators on *Erysimum mediohispanicum* (Cruciferae) fitness. *Oecologia* 143: 412–418. Gómez JM. 2007. Dispersal-mediated selection on plant height in an
- Gomez JM. 2007. Dispersal-inectated selection on plant height in an autochorously dispersed herb. *Plant Systematics and Evolution* 268: 119–130. Gómez JM, Abdelaziz M, Camacho JPM, Muñoz-Pajares AJ, Perfectti F. 2009a.
- Local adaptation and maladaptation to pollinators in a generalist geographic mosaic. *Ecology Letters* 12: 672–682.
- Gómez JM, Abdelaziz M, Lorite J, Muñoz-Pajares AJ, Perfectti F. 2010. Changes in pollinator fauna cause spatial variation in pollen limitation. *Journal* of *Ecology* **98**: 1243–1252.
- Gómez JM, Bosch J, Perfectti F, Fernández JD, Abdelaziz M, Camacho JPM. 2008. Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference patterns of its local pollinators. *Proceedings of the Royal Society of London. SeriesB: Biological Sciences* 275: 2241–2249.
- Gómez JM, Muñoz-Pajares AJ, Abdelaziz M, Lorite J, Perfectti F. 2014. Evolution of pollination niches and floral divergence in the generalist plant *Erysimum mediohispanicum. Annals of Botany* 113: 237–249.
- Gómez JM, Perfectti F, Bosch J, Camacho JPM. 2009b. A geographic selection mosaic in a generalized plant–pollinator–herbivore system. *Ecological Monographs* 79: 245–263.
- Gómez JM, Perfectti F, Jordano P. 2011. The functional consequences of mutualistic network architecture. *PLoS ONE* 6: e16143.
- Gómez JM, Zamora R. 1999. Generalization vs. specialization in the pollination system of *Hormathophylla spinosa* (Cruciferae). *Ecology* 80: 796–805.
- Griffin CAM, Eckert CG. 2003. Experimental analysis of biparental breeding in a self-fertilizing plant. *Evolution* 57: 1513–1519.
- Hardy OJ, González-Martínez SC, Colas B, Fréville H, Mignot A, Olivieri I. 2004. Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae). II. Correlated paternity within and among sibships. *Genetics* 168: 1601–1614.
- Herrera CM. 1987. Components of pollinator 'quality': comparative analysis of a diverse insect assemblage. *Oikos* 50: 79–90.
- Herrera CM. 1989. Pollinator abundance, morphology, and flower visitation rate: analysis of the 'quantity' component in a plant-pollinator system. *Oecologia* 80: 241–248.

- Herrera CM. 2000. Flower-to-seedling consequences of different pollination regimes in an insect-pollinated shrub. *Ecology* **81**: 15–29.
- Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50: 346–363.
- Hurlbert SH. 1971. The nonconcept of species diversity: a critique and alternative parameters. *Ecology* 52: 577–586.
- Inouye DW. 1980. The terminology of floral larceny. *Ecology* 61: 1251–1253.
- Karron JD, Marshall DL. 1993. Effects of environmental variation of singly and multiply sired progenies of *Raphanus sativus* (Brassicaceae). *American Journal of Botany* 80: 1407–1412.
- Kennedy BF, Sabara Ha, Haydon D, Husband BC. 2006. Pollinator-mediated assortative mating in mixed ploidy populations of *Chamerion angustifolium* (Onagraceae). *Oecologia* 150: 398–408.
- King C, Ballantyne G, Willmer PG. 2013. Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution* 4: 811–818.
- Lander TA, Klein EK, Stoeckel S, Mariette S, Musch B, Oddou-Muratorio S. 2013. Interpreting realized pollen flow in terms of pollinator travel paths and land-use resistance in heterogeneous landscapes. *Landscape Ecology* 28: 1769– 1783.
- Ma Y, Yin G, Gao J, Luo Y-B, Bai W-N. 2018. Effects of distinct pollinators on the mating system and reproductive success in *Incarvillea sinensis*, an annual with large floral displays. *Journal of Plant Ecology* 12: 137–143.
- Maloof JE, Inouye DW. 2000. Are nectar robbers cheaters or mutualists? *Ecology* 81: 2651–2661.
- Marshall DL, Ellstrand NC. 1986. Sexual selection in *Raphanus sativus*: experimental data on nonrandom fertilization, maternal choice and consequences of multiple paternity. *The American Naturalist* 127: 446–461.
- Marshall DL, Folsom MW. 1991. Mate choice in plants: an anatomical to population perspective. *Annual Review of Ecology and Systematics* 22: 37-63.
- Marshall DL, Shaner MGM, Oliva JP. 2007. Effects of pollen load size on seed paternity in wild radish: the roles of pollen competition and mate choice. *Evolution* **61**: 1925–1937.
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* 7: 639–655.
- Mayfield MM, Waser NM, Price MV. 2001. Exploring the 'Most Effective Pollinator Principle' with complex flowers: bumblebees and *Ipomopsis* aggregata. Annals of Botany 88: 591–596.
- Montalvo AM. 1992. Relative success of self and outcross pollen comparing mixed- and single-donor pollinations in *Aquilegia caerulea*. *Evolution* 46: 1181–1198.
- Muñoz-Pajares AJ, Herrador MB, Abdelaziz M, Picó FX, Sharbel TF, Gómez JM, Perfectti F. 2011. Characterization of microsatellite loci in *Erysimum mediohispanicum* (Brassicaceae) and cross-amplification in related species. *American Journal of Botany* 98: e287–e289.
- Ne'eman G, Jürgens A, Newstrom-Lloyd L, Potts SG, Dafni A. 2010. A framework for comparing pollinator performance: effectiveness and efficiency. *Biological Reviews of the Cambridge Philosophical Society* 85: 435–451.
- Ohashi K, Thomson JD. 2009. Trapline foraging by pollinators: its ontogeny, economics and possible consequences for plants. *Annals of Botany* 103: 1365–1378.
- Ollerton J, Rech AR, Waser NM, Price MV. 2015. Using the Literature to test pollination syndromes —some methodological cautions. *Journal of Pollination Ecology* 16: 119–125.
- Paetkau D, Calvert W, Stirling I, Strobeck C. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* 4: 347– 354.
- Paetkau D, Slade R, Burden M, Estoup A. 2004. Genetic assignment methods for the direct, real time estimation of migration rate: a simulation based exploration of accuracy and power. *Molecular Ecology* 13: 55–65.
- Pannell JR, Labouche A-M. 2013. The incidence and selection of multiple mating in plants. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 368: 20120051.

- Paschke M, Abs C, Schmid B. 2002. Effects of population size and pollen diversity on reproductive success and offspring size in the narrow endemic *Cochlearia bavarica* (Brassicaceae). *American Journal of Botany* 89: 1250– 1259.
- Perfectti F, Gómez JM, Bosch J. 2009. The functional consequences of diversity in plant-pollinator interactions. *Oikos* 118: 1430–1440.
- Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity* **95**: 536–539.
- Price MV, Waser NM. 1979. Pollen dispersal and optimal outcrossing in Delphinium nelsoni. Nature 277: 294–297.
- Rader R, Howlett BG, Cunningham SA, Westcott DA, Edwards W. 2012. Spatial and temporal variation in pollinator effectiveness: do unmanaged insects provide consistent pollination services to mass flowering crops? *Journal of Applied Ecology* 49: 126–134.
- Reynolds RJ, Fenster CB. 2008. Point and interval estimation of pollinator importance: a study using pollination data of *Silene caroliniana*. *Oecologia* 156: 325–332.
- Rhodes MK, Fant JB, Skogen KA. 2017. Pollinator identity and spatial isolation influence multiple paternity in an annual plant. *Molecular Ecology* 26: 4296– 4308.
- **Robertson IC, Leavitt H. 2011.** Relative contributions to seed production by floral visitors of slickspot peppergrass, *Lepidium papilliferum* (Brassicaceae). *Arthropod–Plant Interactions* **5**: 379–389.
- Robledo-Arnuncio JJ, Austerlitz F, Smouse PE. 2007. POLDISP: a software package for indirect estimation of contemporary pollen dispersal. *Molecular Ecology Notes* 7: 763–766.
- Robledo-Arnuncio JJ, Klein EK, Muller-Landau HC, Santamaría L. 2014. Space, time and complexity in plant dispersal ecology. *Movement Ecology* 2: 16.
- Rodríguez-Rodríguez MC, Jordano P, Valido A. 2013. Quantity and quality components of effectiveness in insular pollinator assemblages. *Oecologia* 173: 179–190.
- Rostás M, Bollmann F, Saville D, Riedel M. 2018. Ants contribute to pollination but not to reproduction in a rare calcareous grassland forb. *PeerJ* 6: e4369.
- Sahli HF, Conner JK. 2007. Visitation, effectiveness, and efficiency of 15 genera of visitors to wild radish, *Raphanus raphanistrum* (Brassicaceae). *American Journal of Botany* 94: 203–209.
- Schemske DW, Horvitz CC. 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialization. *Science* 225: 519–521.
- Schupp EW, Jordano P, Gómez JM. 2017. A general framework for effectiveness concepts in mutualism. *Ecology Letters* 20: 577–590.
- Simons AM. 2011. Modes of response to environmental change and the elusive empirical evidence for bet hedging. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 278: 1601–1609.
- Stebbins GL. 1970. Adaptive radiation of reproductive characteristics in angiosperms, I: pollination mechanisms. *Annual Review of Ecology and Systematics* 1: 307–326.
- Tur C, Sáez A, Traveset A, Aizen MA. 2016. Evaluating the effects of pollinator-mediated interactions using pollen transfer networks: evidence of widespread facilitation in south Andean plant communities. *Ecology Letters* 19: 576–586.
- Valverde J, Calatayud J, Gómez JM, Perfectti F. 2014. Variación intraestacional en los visitantes florales de *Erysimum mediohispanicum* en Sierra Nevada. *Ecosistemas* 23: 83–92.
- Valverde J, Gómez JM, García C, Sharbel TF, Jiménez MN, Perfectti F. 2016a. Inter-annual maintenance of the fine-scale genetic structure in a biennial plant. *Scientific Reports* 6: 37712.
- Valverde J, Gómez JM, Perfectti F. 2016b. The temporal dimension in individual-based plant pollination networks. *Oikos* 125: 468–479.
- Van Der Niet T, Peakall R, Johnson SD. 2014. Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Annals of Botany* 113: 199–211.
- Vázquez DP, Morris WF, Jordano P. 2005. Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecology Letters* 8: 1088–1094.

- Vázquez DP, Ramos-Jiliberto R, Urbani P, Valdovinos FS. 2015. A conceptual framework for studying the strength of plant-animal mutualistic interactions. *Ecology Letters* 18: 385–400.
- Wang H, Cao GX, Wang LL, Yang YP, Zhang ZQ, Duan YW. 2017. Evaluation of pollinator effectiveness based on pollen deposition and seed production in a gynodieocious alpine plant, *Cyananthus delavayi. Ecology and Evolution* 7: 8156–8160.
- Wang J. 2014. Estimation of migration rates from marker-based parentage analysis. *Molecular Ecology* 23: 3191–3213.
- Watts S, Ovalle DH, Herrera MM, Ollerton J. 2012. Pollinator effectiveness of native and non-native flower visitors to an apparently generalist Andean shrub, *Duranta mandonii* (Verbenaceae). *Plant Species Biology* 27: 147–158.
- Wesselingh RA. 2006. Pollen limitation meets resource allocation: towards a comprehensive methodology. *New Phytologist* 174: 26–37.
- Wilcock C, Neiland R. 2002. Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science* 7: 270–277.
- Willcox BK, Aizen MA, Cunningham SA, Mayfield MM, Rader R. 2017. Deconstructing pollinator community effectiveness. *Current Opinion in Insect Science* 21: 98–104.
- Yasui Y, Garcia-Gonzalez F. 2016. Bet-hedging as a mechanism for the evolution of polyandry, revisited. *Evolution* 70: 385–397.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Plots at the Hoya de Pedraza botanical garden.

Fig. S2 Abortion rates of the fertilized ovules.

Fig. S3 Flower handling and fit of some *Erysimum* mediohispanicum pollinators.

Notes S1 Flower handling and fit of *Erysimum mediohispanicum* pollinators.

Table S1 Pollinator functional groups of *Erysimum*mediohispanicum.

Table S2 Analysis of variance and summary statistics of the parameters characterizing the four most effective pollinator functional groups of *Erysimum mediohispanicum*.

Table S3 Population genetic characteristics of the loci genotyped.

Table S4 Subcomponents of the quantity (QNC), quality (QLC)and genetic (GNC) components.

Table S5 Paired post hoc comparisons of the visitation rate.

Table S6 Paired *post hoc* comparisons of the per-plant number ofcontacted flowers.

Table S7 Paired post hoc comparisons of the seed set.

Table S8 Paired post hoc comparisons of the fruit set.

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Table S9 Summary statistics of the paternity analyses.

Table S10 Paired comparison of the per-fruit diversity of sires (PIE and F_S) and the proportion of seedlings sired by immigrant pollen.

Table S11 Pollination distances.

Table S12 Average pollination effectiveness.

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